Evidence based harm reduction

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EVIDENCE BASED HARM REDUCTION

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Table of commonly used abbreviations

ACBI	-	Antibiotic *Clostridium botulinum* Isolation agar
APSS (-200)	-	Automated Parenteral Sampling System (Model 200)
BADAS	-	Bath Area Drugs Advisory Service
BDP	-	Bristol Drugs Project
CBA	-	Columbia Blood Agar
CBI	-	*Clostridium botulinum* Isolation agar
CZE	-	Capillary Zone Electrophoresis
DASH	-	Drugs and Alcohol Service Hereford
ES-MS	-	Electro-Spray Mass Spectrometry
HPLC	-	High Performance Liquid Chromatography
IDU	-	Intravenous Drug User
NHS	-	National Health Service
NMR	-	Nuclear Magnetic Resonance
PEMBA	-	Polymixin pyruvate Egg yolk Mannitol Bromothymol blue Agar
Sab	-	Sabouraud agar

Glossary of drug use related terms

1ml	-	1ml insulin syringe
Acidifier	-	Any substance of an acidic nature used to help render insoluble base drugs soluble for injection
Citric	-	Short reference for citric acid, a popular acidifier
Cooker	-	The spoon or other implement in which the injections prepared and heated
Crack Cocaine	-	The base form of cocaine, made by heating cocaine hydrochloride with sodium bicarbonate. This form allows the drug to be smoked.
Habit	-	The pattern, or level, of drug use/addiction
Heroin	-	Diamorphine
Gear	-	Street drugs, usually refers to heroin
Paraphernalia	-	Refers to equipment and materials used in the preparation and injection of drugs
Speedball	-	A combined injection of a stimulant (amphetamine sulphate or cocaine) and heroin. In the context of this thesis, the term is used solely to refer to the mixture of crack cocaine and heroin.
Use	-	Term meaning to 'ingest drugs'
User	-	A person who uses drugs
Vit. C.	-	Short for 'vitamin c' and referring to ascorbic acid, a popular acidifier

Definitions with importance to this work

Distilled Water	-	Refers to water produced by a Milli-Q PF Water
Milli-Q System	-	This produces ultra-pure water of a higher purity than 18.2MOhms/cm.
Phlebitis	-	Inflammation of the wall of a vein, painful and tender with surrounding skin feeling hot and appearing red. Commonly develops into thrombophlebitis.
Thrombophlebitis	-	Inflammation of the wall of a vein, with secondary thrombosis occurring within the affected segment of vein
Thrombosis	-	A condition in which the blood coagulates forming an occlusion in the vascular system, commonly the deep veins.

Abstract

Review of the literature demonstrated that knowledge surrounding the process of illicit drug injection preparation is limited. A better understanding of the details of injection preparation methods could inform study of the risks and complications, as well as the development of harm reduction advice.

This project had two main objectives: To characterise the injection preparation process in detail, then to attempt to quantify the risks posed by these injections through laboratory investigations. The project had specific focus on the use of the acids by injectors to increase the solubility of insoluble illicit drugs.

To study injection preparation, a novel interview was designed to record the methods that a cohort of injectors used to prepare their injections of heroin and 'crack' cocaine. The interview incorporated two separate sections: firstly a semi-structured questionnaire, then observation of participants preparing an inert 'fake drug' for injection using their usual preparation procedure for real drug.

The injector interviews documented the use of acids by injectors in detail. The injection preparation demonstration enabled a complete characterisation of the preparation procedures for heroin, crack and speedball injections and enabled the development of a standardised method by which injections using real drug samples could be reproduced in the laboratory.

Prepared injections were subjected to a number of assays to evaluate their properties. These assays allowed a comparison with pharmaceutically prepared injections (pharmacopoeia standards) to provide some quantification of risk. Investigations were conducted into the drug content, the microflora, the particulate content and the physical characteristics of the injections.

Electrospray mass spectrometry was used to identify the components within illicit heroin samples. This methodology has never before been used to examine illicit drug samples.

The project developed microbiological investigation techniques that enabled the isolation and identification of micro-organisms within drug injection solutions.

Particulate content of injection solutions was found to be high, and the use of rudimentary illicit filters was shown to add to this. The use of filters made specifically for illicit drug users could significantly reduce this content.

Overall, no significant risks were identified and this supports current harm reduction advice which was not based on any published research.

The author of this thesis performed all data collection except for osmolality investigations. The author undertook all data analysis and production of this thesis. Statistical support was provided by Dr Gordon Taylor, a statistician from the University of Bath, where required.

Thesis Structure

The format of this thesis follows the order in which the work it documents was conducted. The thesis is divided into two main sections; first is the interview work and its analysis with discussion. Secondly, the experimental section of the work details the work conducted using injections prepared and studied within the laboratory environment. The thesis concludes with an overall reflection on contribution to knowledge from this work.

1 Literature Review

1.1 Literature search strategy

The references for the following literature review were obtained from searches of the databases of Web of Science, MedLine and Embase. A large variety of search terms were used, alone and in combination, to build a reference base for the study.

Overall, there were a very limited number of references in this specific area of study. Additional information was gathered from various sources outside of peer-reviewed journal literature, including books, drug user information leaflets, and the internet, specifically the World Wide Web, and 'usenet' (the oldest type of electronic communication, after email, comprising of 'Newsgroups'). Anecdotal reports and personal communications are also included where their inclusion is of importance. All sources are referenced.

1.2 History of drug use and injecting

1.2.1 Drug use

There is no clear or simple explanation of why people use illicit drugs for recreational purposes. By changing the awareness of the user, drugs produce feelings of well-being or euphoria- commonly described as the 'high'. This explanation provides one obvious reason, but there can be more complex motivations. Weil and Rosen (1993) list many reasons why people use drugs to change their awareness, including: to aid religious practices, to explore the self, to escape boredom and despair, to promote social interaction, to stimulate artistic creativity, to rebel, peer pressure, or to self treat illnesses.

Cannabis is the most commonly used drug in British society (Home Office 2005), and people from diverse backgrounds choose to use it. It is used for a range of reasons including socially, for relaxation, for religious purposes,

and to ease pain. To this effect it has been suggested that it can become dependence forming (Farrell 1999). The choice of drug will depend on its pharmacological actions. Many drugs fit in with the social factors present in the user's life. For example, ecstasy and amphetamine are commonly used by young people, who only use them occasionally and only when they are going to dance music events. Glue, lighter fluid and other volatile substance sniffing is popular with young school-age children.

Modern increases in wealth and technology have brought homelessness and unemployment, which are two common factors seen amongst drug users. Prosperity has also lead to the rise in commercial imports and personal travel over the last century. Both of these have increased the availability of illicit drugs; heroin is a prime example of a drug in this category. Imports of the drug have grown dramatically in the past twenty-five years. Despite apparent increased customs seizures, prices have fallen, purity levels have increased and the drug is more easily available than ever (EMCDDA 2001). Heroin is suited to use by people who are unemployed, it can fill the void in their life, blocking out social and financial worries, but its use is not limited to people of this status.

Drug use is an emotive issue. It affects not only the drug users, but society as a whole, through crime, anti-social behaviour and associated depravation. Taxation funds the NHS which provides treatment for the effects and complications associated with drug misuse. Reducing the need for these interventions would be of benefit by enabling the NHS to use the funding for other purposes. Investigation into the causes of these health complications could prevent them occurring in the first instance.

Harm Reduction is a philosophy which aims to lower the harm drug users suffer through the use of illicit drugs recognising that cessation of the drug use may not be immediately achievable due to various factors motivating the continued drug use and dependence. Harm reduction interventions aim to protect the drug user and society from the risks associated with illicit drug use.

1.3 Drug injecting

The practice of injecting drugs was established by the medical profession. Soon after, the injection of drugs for non-medical purposes began to

occur. By the beginning of the twentieth century, the injection of heroin and cocaine for recreational purposes was common on both sides of the Atlantic Ocean (Karch 1996). The development and refinement of injecting equipment, combined with increased availability of illicit drugs throughout the twentieth century, led to a continued popularity in illicit drug injecting. From its initial stages in developed countries, the process of injecting spread globally. Drucker et al.(2001) estimated that there are between 10 and 15 million people who inject illicit drugs worldwide, in more then 120 countries. This figure is likely to grow if drug use continues to increase.

1.3.1 Illicit drug injection

Drug users choose to use drugs by injection for numerous reasons. Factors encouraging the use by injection include the reduction in wastage, making the drug more cost effective, and because of the euphoric sensations produced on injection.

The wastage of a drug when used by some routes is clear. When smoking heroin for instance, it is obvious that a certain amount will escape being inhaled. Additionally, good technique is crucial to achieve optimum effects, as over heating can result in breakdown of the drug instead of it being vapourised. Injecting heroin users are seen to use less heroin than those who smoke it (Huizer 1987).

The initiation of new injectors is important for the continued existence of an injecting drug population (Crofts et al. 1996). Witnessing friend's reactions to injections of heroin can tempt non-injectors to move to injecting. Stewart (2002) gives a comprehensive account of users feelings towards and against initiating others into injecting, as well as giving insight into the users psychological reasoning and considerations at the time of initiating others.

1.3.2 Drugs injected illicitly

In the United Kingdom at the current time, the most commonly injected drugs are heroin, amphetamine and cocaine (Bennett 1998). However, the injection of cocaine derived from 'crack' is increasing in popularity (Hunter, Donoghoe, & Stimson 1995).

Almost all drugs of abuse have reportedly been injected at some time, including: amphetamine/methamphetamine, barbiturates, benzodiazepines (Robertson et al. 1987;Ross, Darke, & Hall 1997), cocaine, crack, cyclizine (Rubin et al., 1989), Diconal®(Marjot 1978), ketamine, LSD, MDMA (Ecstasy) (Wafer 2001), opioids (including methadone, pethidine, buprenorphine and fentanyl), opiates (including heroin, morphine and codeine) and phencyclidine (PCP).

Alcohol is reported to have been injected (Derricott, Preston, & Hunt 1999), 'dope' [referring to cannabis] (McBride et al. 2001) and even morning glory seeds (a hallucinogen containing LSA), have been boiled up to produce an injectable solution (Fink, Goldman, & Lyons 1966). Non drugs have also been injected, including victory V sweets (McBride, Pates, Arnold, & Ball 2001). It seems the availability of injecting equipment, namely a needle and syringe, tempts some users to inject anything to explore the effect it may produce.

When the users run short of drug, some resort to various methods to attempt to induce further intoxication. McBride et al. quote two common habits-injecting water, and injecting a solution prepared by soaking used filters. The injection of water can produce no more than a 'placebo effect', and potentially demonstrates, to an extent, the psychological component involved in the actual effect of the injection process.

Although there have been no investigations into the drug content of used filters, residual fluid within used filters will contain drug. Soaking one or more used filters in water will enable the extraction of a portion of the drug into the water. This is potentially a highly hazardous activity. The filters will have been stored since they were first used; this could have been on a table top within an injecting environment, or in a dirty pocket. After the initial use, filters will be warm and damp, providing suitable environments for bacterial and fungal growth. These organisms may then pass into the injection solution and be injected. There is also the possibility of blood-borne viral infection if the filter was contaminated during its previous use.

1.4 Injecting complications

The injection of *any* drugs, medical or illicit, can lead to health complications. The nature of the injection process is inherently risky as it

circumvents the body's natural defences, introducing materials including drugs, inactive substances, particulate matter and micro-organisms directly into the body. Drucker et al. (2001) account how the proliferation of the use of injections in the Twentieth century has led to epidemic infections of hepatitis B, C and HIV amongst IDUs.

Complications also occur due to the nature of the injection solution and the manner of the injection. Extravasation of cytotoxic drugs used under medical circumstances can be extremely dangerous. This results from a combination of drug irritancy and administration problems. Some drugs are simply unsuitable for injection, even in pharmaceutical injectable preparations. Langdon, Harlan, & Bailey (1973) reported the medicinal intravenous injection of diazepam causing thrombophlebitis. Diazepam misuse is common, and injection of crushed tablets has been reported (Ross, Darke, & Hall 1997). It is therefore likely that the injectors are exposed to problems posed by the diazepam, in addition to those posed by an illicit injection of an oral preparation.

Three main factors determine the risk an injection poses. Firstly, the injection itself, secondly, the preparation method and the environment under which it takes place and finally, with respect to illicit drug use, the social circumstances under which the injection is prepared.

1.4.1 Causes of complications

Complications are encountered in the use of pharmaceutical preparations in medical use, but the illicit preparation and injection introduces further problems.

Injections performed by untrained people, can lead to problems due to fundamental lack of understanding regarding injection administration, for instance, injecting into arteries instead of veins. This can result in arterial spasm, dangerous heavy bleeding, or gangrene as a consequence of particulate matter occluding blood vessels.

The injection of preparations not intended for injection, including pharmaceutical formulations made for the oral route, poses hazards. Illicitly produced drugs are likely to be unsuitable for injection, as they contain unknown quantities of drug, impurities, adulterants and micro-organisms.

The reuse and sharing of injecting equipment introduces further risks and complications. Viral transmission of HIV and hepatitis through sharing of

injection equipment is well documented. Repeated use of needles results in them blunting, leading to physical damage to veins which does not occur with new sharp needles. The end of the needle can form a barb shape which tears tissue upon use, especially as it is withdrawn. As outlined above, the storage and reuse of filters may facilitate the growth of bacteria and fungi, which could enter injection solutions into which the filter comes into contact.

The environment under which an injection is prepared, and subsequently administered has an important impact upon its risks. Dirty, unsanitary conditions such as public toilet cubicles, outside locations, or unclean housing are likely to introduce risk of contamination of the injection itself and/or the equipment, leading to problems such as infections. The presence of animals, such as pet dogs, in the immediate environment will add to these risks.

The condition of the injector is also important. If the user is intoxicated, or withdrawing at the time of injection, harm may increase. Withdrawal can make the user shake and sweat, making it more difficult for them to perform the injection. The desire to get the drug into their system as soon as possible may force them to rush the injection, leading to problems such as missing the vein, leading to injection extra-vascularly. Intoxication can lead to lack of coordination and poor judgement. Repeated injections of cocaine at the same site can lead to tissue damage and missed veins; the local anaesthetic nature of the drug means the injector is unaware of pain that would normally warn them of problems. Cocaine has a short half-life- approximately 41 minutes when given intravenously (Javaid et al. 1983). This has implications for IDUs as it can drive frequent injecting. This leads to greater physical damage to veins and increased possibility of infections such as abscesses, or blood borne viruses from sharing equipment.

1.5 Harm reduction and the injecting drug user

Harm reduction is a set of policy beliefs, essentially accepting that some people always have and always will perform activities, such as promiscuous sex or drug use that may cause harm. Therefore, there is a moral imperative to reduce the harm caused by these risky activities, rather than an ineffective blanket prohibition of the harmful activities.

With regard to injecting drug users, harm reduction in its most obvious form exists as needle exchange. In order to reduce the spread of blood borne viruses, as well as reduce infections caused by using non-sterile injection paraphernalia, needle exchanges provide sterile equipment to injectors.

The supply of clean injecting equipment is only one area of harm reduction. The adoption of safer injecting rooms, education sessions about injection techniques and the testing of drug material would arguably provide further benefits for injecting drug users (IDUs). Although proposals for these have been made, they remain to the large part only as model ideals.

In response to calls for changes, the British government recently reviewed the Misuse of Drugs legislation with the intention of improving services involved in harm reduction work with drug injectors (Home Office 2001). Research into the injection preparation methods in use by IDUs will be important in the planning these changes required to services. Research into methods of drug use this will provide the evidence basis on which to inform and implement these changes.

1.6 The preparation of illicit drugs for injection

1.6.1 Literature documentation of injection preparation techniques

There is very little work in the literature documenting the processes of preparing illicit drugs for injection. The majority of information available takes the form of anecdotal details collected in order to enable the production of safer injecting guidelines for drug users. Literature searches revealed only two papers to have studied this process, written by Scott et al.(2000) and Strang et al. (2001). The nature behind the study by Strang was to ascertain which 'acids' were favoured for use by the group of respondents in London. Participants were also asked about the need to heat the mixtures with regard to the acids they used. The paper did not investigate the entirety of the method of preparation, but focussed specifically on the decision to use acid and heat in the preparation. No quantitative data was recorded such as the amounts of drug, acid and water, nor was there any detail about the heating process, such as when heating was stopped and why. The work of Scott was the first to outline the complete

preparation procedure of heroin injections. It records those used by a cohort of injectors in Scotland.

A generic outline of the process that drug injectors are advised to follow to prepare an injection is given below. It has been compiled with information from various sources including: the Safer Injecting Briefing (Derricott, Preston, & Hunt 1999), Scott et al.(2000), various safer injecting leaflets from the agencies Lifeline (2001) and HIT (2001), and additionally a user guide on the web ('Brown Addict' 2002).

The lack of in-depth process details, such as quantities, becomes clear when reading these literature sources. None of the documents give a complete insight into how users prepare drugs. Furthermore, the reliability of the data for the most part is unclear. This is due to the lack of peer-reviewed work in this area- most documents available being of a basic nature written solely as safer injecting guidelines for drug users.

The actual method an injector follows is variable and will depend on many factors, such as the nature of the drug they are using, the choice and availability of materials and equipment, the influence of the people who taught them to prepare injections, along with that of their current drug using peer group, and their own individual habits.

1.6.2 Generalised Injection Preparation Procedure

Items used:

Drug
Water
Acidifier
Needle and syringe
Filter material such as cotton wool
Object to mix and heat the solution such as spoon, sometimes referred to as a 'cooker'
Heat source, such as a matches or a cigarette lighter
Knife or other implement to measure drug and acid amount

General procedure outline:

1. Drug is taken out of container, measured to desired dose, and placed in cooker
2. Needle is attached to syringe
3. Water is drawn up with syringe and added to cooker
4. Mixture is stirred
5. If drug doesn't appear to dissolve (solution remains cloudy), add acidifier in smallest quantity necessary
6. Heat is applied if needed for a short time
7. Filter is placed in solution
8. Solution is drawn up into syringe through the filter
9. Syringe inverted and excess air expelled
10. Injection takes place

Many regional and personal variations of the above process are likely to exist, with additions and omissions to this routine, possibly leading to the existence of a large number of significantly different techniques. Whether any of these methods is safer than any other is an important question.

The above description immediately raises issues if it is to be replicated for investigation. For instance, how much water should be used? What size needle and syringe? How long is the preparation stirred for, and what with? How much acid should be used?

This presents an interesting research question: *Exactly* how are injections prepared by injectors? To investigate the dangers posed by the injections it is imperative to understand the nature of the injection preparation and the materials from which it is prepared. Specific detail is necessary in order to develop a reproducible laboratory method.

1.7 Heroin and cocaine use

Heroin (diamorphine) is used in the United Kingdom for analgesia and the treatment of acute pulmonary oedema (BNF 2002). The main reason for its use, especially in consideration to its main alternative morphine, is the high solubility of diamorphine hydrochloride in water. This allows administration of

high doses in small volumes, making injections easier and less painful. Cocaine is rarely used now except as a topical application in otolaryngology.

1.7.1 Illicit heroin and cocaine

Since 1978-79, the majority of the heroin on the illicit market in the UK and mainland Europe has been of the base form (Griffiths, Gossop, & Strang 1994). There are a number of suggestions why this is the case; it is possible because conversion to the hydrochloride salt would add an extra stage to the manufacturing process. Alternatively, the base form is produced as it allows smoking of the drug. This predominance of the base form of heroin has allowed the smoking of heroin to become more common, as it is more volatile (Huizer 1987). Prior to this predominance, heroin was available as the hydrochloride salt, referred to as 'Chinese heroin', it was white in colour. The users of this type of heroin injected it because it was highly soluble in water and smoking this salt form results in only small quantities of the drug in the smoke, as it has a higher melting point (Huizer 1987). The higher temperature required to make the hydrochloride form volatile results in a large degree of pyrolytic degradation of drug molecules (Cook & Jeffcoat 1990).

The visual appearance of these two forms of illicit heroin is shown in Figure 1 below. The colour difference between the base form (brown) and the hydrochloride salt (white) is clear.

Figure 1 The two different forms of illicit heroin: brown heroin base (left) and white heroin hydrochloride

Unlike pharmaceutical grade products, the quality of the drugs on the illicit market is highly variable. This is especially true for drugs in powder form,

where other powders may have been mixed in with the powder to bulk up the quantity (Coomber 1997), or to improve characteristics, such as the volatility when smoked (Huizer 1987). Along with this, the quantity of the drug in illicit powders will also vary depending on a number of factors such as the quality of the raw material from which the drug was originally made, the manufacturing process used and how well the process was performed.

Cocaine in the form of 'crack' has been commonly available since the early to mid 1980s (Jones 1990). Before this time, cocaine was available as the hydrochloride salt and in the form of freebase. Freebase was rarely found and was usually produced directly by the users themselves from the hydrochloride salt. Crack and freebase are essentially the same as they are both the base form of cocaine. Crack is produced using sodium bicarbonate powder, and some of this remains in the final product, freebase is formed using ammonia liquid, which is not retained in the final product. Both freebase and crack are formed to enable the smoking of cocaine, as it cannot easily be smoked as the hydrochloride salt. The base form has a low melting point (80°C, (Jones 1990)) meaning the drug is more volatile. Increases in the popularity of crack/freebase have resulted in some users choosing to inject it.

1.8 Solubility

For a drug to be injected, its solubility in water is important. If the drug will not dissolve this will pose a problem- if a drug is not soluble it is unlikely to be used by injection.

Of the three main drugs injected illicitly: amphetamine is found on the illicit market is as the sulphate salt. This is freely soluble in water and makes it easy to prepare for injection. Cocaine hydrochloride powder also has a high solubility, as does pharmaceutical diamorphine hydrochloride, but illicit heroin and 'crack' cocaine are not so easy to use.

The difficulty with injecting 'street' crack cocaine and the majority of illicit heroin (Strang, Griffiths, & Gossop 1997), is that neither of them are particularly soluble in water. As previously outlined, both drugs are in the base forms. Heroin base is highly volatile, and therefore is ideal for smoking (Huizer 1987), but for injecting users, it presents a problem by being so poorly water soluble- 1gram in 1700ml (Clarke 1986). Cocaine as 'crack' is the base form of the drug in a bicarbonate crystalline mixture (Jones 1990) and this too, is poorly soluble

in water- 1 in 600ml (Clarke 1986). In comparison, the cocaine hydrochloride from which the crack is manufactured, is very soluble- 1 in 0.5 of water (Martindale 1999), 1 in 0.4 (Merck Index (2003a)).
Tables 1 and 2 show the solubility of diamorphine and cocaine respectively.

Table 1. Diamorphine solubility

Solvent	Base Solubility	Hydrochloride solubility	Reference
Water	1g in 1700ml	1g in 2ml	Clarkes Isolation and identification of drugs (Clarke 1986)
Ethanol	1g in 31ml	1g in 11ml	
Ether	1g in 100ml	Practically insoluble	
Chloroform	1g in 1.5ml	1g in 1.6ml	

Table 2. Cocaine solubility

Solvent	Base solubility	Hydrochloride solubility	Reference
Water	1g in 600ml	1g in 0.5ml	Clarkes Isolation and identification of drugs (Clarke 1986)
Ethanol	1g in 7ml	1g in 3.5 to 4.5ml	
Ether	1g in 4ml	Practically insoluble	
Chloroform	1g in about 0.5ml	1g in 15 to 18ml	
Water	1g in 600ml (270ml at 80°C)	1g in 0.4ml	Merck Index (2003a)
Alcohol	1g in 6.5ml	1g in 3.2ml (cold), 2ml (hot)	
Ether	1g in 3.5ml	Insoluble	
Chloroform	1g in 0.7ml	1g in 12.5ml	

This low solubility presents a problem for users when preparing to inject these forms of the drugs. To render these drugs soluble for injection, users have to convert the base form to an acid-salt form, which increases the solubility. To convert diamorphine or cocaine base bought illicitly, injectors utilise acids that are easy to obtain.

1.8.1 The chemistry behind solubility

Not every chemical is soluble in every other chemical. In order for a substance (*the solute*) to be soluble in another (*the solvent*), it must possess the correct chemical properties. This is usually expressed in a simple manner as 'like dissolves like'. As the base forms of the drugs do not dissolve in water, it is apparent that they are in the wrong state.

Water is a 'polar solvent' as it has a permanent dipole moment. The base forms of the drugs are the 'non-polar' forms. They do not ionise to any great extent when in water. The base forms of drugs are soluble in non-polar solvents, such as ether, but poorly soluble in polar solvents such as water.

In order for basic drugs to be dissolved in water they need to be converted to a polar form. This is done by converting them to an *acid-salt.* To form the *acid-salt*, drug injectors react the drug base with an acid. This means that when the drug is added to water it can dissociate, or split, into an ionised form which is soluble in water. The drug becomes ionised by accepting a proton (H^+) from the acid the salt was formed with, giving the drug molecule a positive charge.

$$\text{Drug Base} + \text{Acid} \longrightarrow \text{Drug}^+ + \text{Acid Salt}^-$$

This charge enables the drug molecules to dissolve into solution as the water molecules are able to surround each drug molecule individually, thus dissolving it.

Depending on the acid used, and its propensity to dissociate (quantified by its pKa value), an acid molecule may donate different numbers of H^+ ions. If fully dissociated, ascorbic acid can donate two protons, and citric acid can donate three.

The pharmaceutical forms of heroin and cocaine are supplied as the hydrochloride salts which are soluble because they accept an H^+ ion from the hydrochloride molecules.

Injectors could use hydrochloric acid to produce the hydrochloride salt of illicit drugs, but it is difficult to obtain, and its use would pose hazards during the preparation and injection. In order to form the salt, injectors use acids that are easy to obtain, such as vinegar, or lemon juice.

The use of vinegar (ethanoic acid) will produce the diamorphine ethanoate salt. Citric acid will react with diamorphine base to produce diamorphine citrate. Vitamin C (ascorbic acid) will produce the ascorbate salt.

1.9 Acidifiers

The use of acidifiers by injecting drug users is confined to regions where the drugs are supplied as the base forms. In the UK, the majority of heroin and crack require acidifiers to be utilised in the preparation of an injection. In North America and Australasia the majority of heroin on the illicit market is the hydrochloride salt form and therefore it does not require conversion to an acid-salt. This means that in these regions, only crack requires the use of an acid during injection preparation. Due to crack injection generally being an uncommon practice, the preparation process involving acid-salt conversion is much less widely known in these regions (Kinzly 1997). This means people who attempt to prepare crack for injection use vinegar (ibid) rather than a purer forms of acid such as citric acid. In the UK, people who choose to inject crack may have injected heroin previously so they are familiar with the use of acids. Alternatively, heroin or crack injectors within their peer group may educate them about the process of adding acid when preparing these drugs.

1.9.1 Acidifiers used

Drug users have been reported to utilise many various acids to be used as 'acidifiers'.

Commonly reported acidifiers used include:

- Citric acid (Roberts & Thomson 2000;Scott et al. 2000;Strang et al. 2001)
- Ascorbic acid (Vitamin C powder) (Scott, Winfield, Kennedy, & Bond 2000;Strang, Keaney, Butterworth, Noble, & Best 2001)
- Lemon juice (Gallo et al. 1985;Hoy & Speed 1983;Page & Fraile 1999;Shankland & Richardson 1989;Shankland, Richardson, & Dutton 1986;Strang, Keaney, Butterworth, Noble, & Best 2001)
- Vinegar (Scott & Bruce 1998;Strang, Griffiths, & Gossop 1997)

Less commonly used acidic substances/products:

- Baby bottle sterilizer and Kettle descaler (Roberts & Thomson 2000)
- Various effervescent tablets (Preston, Derricott, & Scott 2001)
- Powdered health drink sachets (Roberts & Thomson 2000)
- Fizzy coatings from children's sweets (Derricott, 2002, Personal communication)

A wide range of substances can be used as acidifiers. There are no standards of which one to use, nor, how much should be used, as nothing is designed to be used for this purpose. This situation has left drug users very vulnerable, and results in complications.

Injections prepared using lemon juice and vinegar have been implicated in the cause of Candidal endophthalmitis (Shankland, Richardson and Dutton, 1986) and fungal endocarditis (Bisbe et al. 1987).

The work by Strang et al. (2001) gave information on the prevalence of particular acid use by heroin injectors. They found that for the majority of the time most of the users preferred to use either Vitamin C powder (47.1%) or citric acid (54.8%). Less popularly used were vinegar (2.9%) and lemon juice (1.9%). 2.9% of users reportedly used no acid at all. The use of other acidifiers is most likely rarer, probably confined to specific geographical regions where it is difficult to obtain acids that would normally be preferred.

1.9.2 Choice of acidifier

The reasons why users choose one acidifier in preference to another are unknown, and it is possible that are not specific preferences.

Suggestions for use of one acid over another:

- Works better (in their opinion)
- Less pain on injection
- Perceived danger in use
- Peer pressure/advice
- Availability

1.10 Acidifiers in detail

This section details the acidic substances most commonly reported to be used by drug injectors. It briefly outlines each acid, describing its content, its method of manufacture, its usual uses, and its biological role (if any). It also gives some speculation on possible consequences of parenteral use.

1.10.1 Citric Acid

Use by drug injectors

This is one of the most commonly used acid by injectors as it is currently the one that is recommended for use by most safer injecting guidelines, including the most comprehensive currently available, The Safer Injecting Briefing (Derricott, Preston, & Hunt 1999). For this reason, it is supplied to drug users at needle exchanges in many parts of the country. The safety of using citric acid to prepare injections has never been assessed.

Nature of substance and manufacture

The theoretical reasoning for the safety of this acid is its natural abundance in the human body and its integral part in the process of cell respiration in the Krebs cycle- also known as the citric acid cycle.

Citric acid was first extracted from lemon juice in 1874. It has been used in foods for over one hundred years and is used in many roles including, as a sharp-tasting flavouring, as a preservative and in the cheese making, wine and brewing industries. The range of foodstuffs it is found in is vast. The Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) formed the joint FAO/WHO Joint Expert Committee on food additives (JECFA), which issues monographs and other details on the use of food additives. These include recommended acceptable daily intakes of such additives. In the case of citric acid, they state that it can be used up to a level of 1% of the total mass in

foods, and they give it an unlimited daily intake level (Doull, Klaassen, & Amdur 1980).

Typical uses

Medically, citric acid has been used for the treatment of xerostima, to dissolve renal calculi, alkalinise the urine, in rectal enemas for constipation and to prevent the encrustation of urinary catheters. It has also been used in preparations for the treatment of gastro-intestinal disturbances and metabolic acidosis (Martindale 1999).

Citric acid is used to produce the effervescence in some mixtures and tablets. This has led to the use of such products by some injectors to dissolve heroin and crack for injection. This was particularly observed in western Scotland up until 1999, involving a health drink called 'Abdine' (Roberts & Thomson 2000).

Citric acid is available as large boxes of 'culinary grade' powder, which are commonly purchased from pharmacies. Until the changes in the law that allowed the sales of citric acid (Section 1.11.2), sales of citric acid from pharmacies were discouraged under ethics guidelines (Royal Pharmaceutical Society of Great Britain 2001). Despite the changes in the law some pharmacies still refuse the sale of citric acid, either through ignorance or habit (Personal observation and (Scott 2005b). Citric acid is also available for purchase from Asian food shops. This is of unknown quality.

Citric acid is now available in sterile 100mg sachets, specifically for illicit injection preparation, from a number of suppliers.

Safety and Risks

Even though citric acid is commonly used by injectors, this does not mean it is safe to use. 'Burning' down the limb when using injections prepared with citric acid is commonly reported (Scott & Bruce 1998).

1.10.2 Ascorbic Acid

Use by drug injectors

This is usually purchased in the form of Vitamin C preparations, especially the powder (Preston & Derricott 1999). This being a commonly used acidifier, it was also listed in the Medicines, Ethics and Practice guide for pharmacists, alongside citric acid, to discourage sales. Recent changes have legalised the supply of ascorbic acid to injectors (2005b). Like citric acid, it is also available in sterile sachets (300mg) for supply from needle exchanges facilities.

Nature of substance and manufacture

Vitamin C is a vital constituent of the human diet, as unlike most other animals, humans are unable to synthesise it. It is required for the synthesis of collagen and intracellular material, as well as performing important antioxidant roles. Deficiency leads to scurvy.

Typical uses

To treat deficiency, vitamin C can be given orally as ascorbic acid tablets or as a fruit juice mixture. It can also be injected by the IV, IM or SC routes, as sodium ascorbate.

Safety and Risks

The use of ascorbic acid to prepare injections may lead to complications depending on its source. Pharmacy restrictions have made it difficult to obtain it in the pure powder form. This may have led to the use of Vitamin C tablets. The use of these will involve the injection of the ingredients used to form the tablets, for instance, lubricants, bulking agents and disintegrants.

1.10.3 Vinegar

Use by drug injectors

The main reason for the use of vinegar by drug injectors is the acetic acid content of the solution. Ethanoic (acetic) acid makes up 4-5% of the active ingredient in vinegar (Van Nostrand 1995).

Nature of substance and manufacture

Malt vinegar is usually made from malted barley and other cereals. Appropriate yeasts are used to ferment sugars in the cereals to ethanol and carbon dioxide. The yeasts are then removed and the alcoholic solution is treated with *Acetobacter sp.* bacteria, which acetify the alcohol. The resulting solution is then filtered and pasteurised to produce clear vinegar, which is then left to mature. This is commonly distilled to produce 'distilled malt vinegar' and this contains only the volatile constituents of malt vinegar.

'Non-brewed condiment' is a popular alternative to vinegar. This is a solution of about 4.5% m/v synthetic acetic acid, which is coloured using caramel.

Brewed vinegars can contain significant proportions of B vitamins including thiamine, riboflavine, nicotinic acid, pantothenic acid and pyridoxine (Kirk & Sawyer 1991). These vitamins are absent from the non-brewed products.

All vinegars and artificial alternatives are subject to tests for the following: total acidity, ash, preservatives, arsenic, copper, lead, zinc, alkaline oxidation value, iodine value, and a qualitative test for mineral acid. It is also subject to organoleptic examination looking at the odour and taste of the product.

Vinegar can be tainted with growths of vinegar eels (*Anguillula aceti*), which are slender, aquatic nematode worms which grow up to 2mm in size. These live in dilute wine and/or vinegar and water solutions. Vinegar can be tainted with other micro-organisms, so it is common to find it has preservatives added. Sulphur dioxide and sorbic acid, a polyunsaturated fat, are most often used for this purpose.

Typical uses

Vinegar is commonly used in food preparations, particularly in vinaigrettes, in pickling processes and it is a condiment.

The purpose of using vinegar to acidify drug mixtures is due to the production of the ethanoate salt of the drug. Reaction with diamorphine base in heroin powder will produce the salt form, diamorphine ethanoate.

Administration into the body will lead to the ethanoate section of the molecule entering the citric acid cycle, and being excreted as water and carbon dioxide after the process of cellular respiration.

Safety and Risks

The use of vinegar and non-brewed condiment for the purpose of preparation injections is likely to be hazardous due to the non-sterile nature of the solution and due to the additional chemicals present. The use of these acids from large containers makes them liable to contamination during use from bacteria and fungi, and possibly viral contamination introduced by other injectors sharing the same container.

1.10.4 Lemon Juice

Nature of substance and manufacture

This is obtained by straining the juice pressed from fresh lemons. Of 100g of the juice, 91.3g is water, 1.6g is sugar, 0.3g protein according to McCance and Widdowson (Paul & Southgate 1978). There are also small amounts of the B vitamins thiamine, riboflavin, nicotinic acid, and pyridoxine present (ibid).

Fresh lemon juice contains between 40 and 60mg of vitamin C per 100g.

Lemon juice available in the UK is usually obtained in the form of Jif® lemon. This is made by reconstitution of the frozen concentrate (Personal Communication, Reckitt Benckiser, 2002). The citric acid content of the reconstituted form is stated as 'not less than 5.0grams per 100ml of reconstituted lemon juice' (U.S.Department of Agriculture 1998).

Therefore, lemon juice contains two acids that are useful in the acidification of heroin- ascorbic and citric. Due to its higher concentration, citric acid is arguably the more significant of these two.

Typical uses

Lemons are used in cooking, and as a garnish in drinks. Lemon juice is added to foods, such as pancakes, and fish dishes where the acidic nature of the juice neutralises the taste of the amines.

Use by drug injectors

Page and Fraile (1999) described the use of lemon juice by injectors in Valencia, Spain, as being a unique activity. On the contrary, this method of use has been reported in the UK and Australia since the late 1970s, after being implicated in various infections. It is likely that the main author of the article, who is based in Miami, was unfamiliar with the practice due to the general absence of heroin base on the US illicit market, and hence the lack of need to use an acidifier in injection preparation. The paper shows little understanding of the chemistry of different salt forms of heroin and indeed of heroin itself- even stating that heroin reacts with citric acid to produce 'morphine citrate'. The paper that Strang et al. produced in 2001 was in part a response to the publication of Page et al.. They too refute the conclusions made in the former. Strang et al. interviewed 104 people all of whom had used the common 'brown' heroin that requires acidification- 103 of the 104 (99%) had used acid with brown heroin on at least one occasion. Referring to the last injecting occasion (n=98), 3 people (3.1%) had used lemon juice to prepare it. Overall 89 (85.6%) had used lemon juice at some time and of these 82 (92.1%) applied heat. Of these 89, 38 (36.5%) had used fresh lemon, 23 (22.1%) had used Jif® and 26 (25%) had used both.

In contrast to the brown (base) heroin, they report that the white heroin of the salt form is also found in London. This is much more soluble and likely to dissolve in water by just applying heat. When using white heroin (n=79), the number of people using an acidifier at any time was much lower, 28 (35.4%). Of these, only 15 (14%) had ever tried to use lemon juice; 11 would also heat the mixture.

These are the only figures that can be found in the literature regarding the prevalence and details about the use of lemon juice to prepare injections. It shows that it is rarely the acidifier of choice, but most users had used it at some point. The information distinguishing lemon juice from juice obtained from a fresh lemon is also important. The juice from a freshly cut lemon fruit is unlikely to contain bacterial or fungal organisms (but it is possible for organisms to be introduced into the juice during the cutting and squeezing process).

The interviewers also recorded the reasons why users did not regularly use lemon juice. The majority said it didn't work, but some were aware that its use had been linked to infections.

1.10.5 Lemon juice preservation and the possible link to infections in injectors

Due to its acidity (pH range 2.2-2.6), lemon juice is largely unaffected by bacterial spoilage, but it is prone to fungal spoilage, these organisms being more tolerant of low pH values (Splittstoesser 1978). To prevent this, the manufacturers of Jif® lemon juice add sulphur dioxide to the solution. Over time, however, exposure to the air and warm temperatures, leads to this gas gradually coming out of solution. This leaves the juice a viable medium for fungal growth. These organisms will then pose a potential risk if this solution is used for injection preparation. Repeated opening of the container to acquire juice will increase the risk of the solution within the container becoming contaminated. Combined with a lack of refrigeration, this will provide a suitable environment for the growth of fungal organisms.

1.11 The role of acidifiers in health complications

The use of acidifiers to prepare heroin and cocaine for injection introduces an additional component to further the risks posed by prepared injections.

1.11.1 Acids and vein damage

The injection of solution into veins, especially small veins as found in the hands and feet, can lead to problems if the pH and osmolality of the solutions are very different to that of biological fluids. Even in pharmaceutical preparations it is difficult to get the characteristics of a solution similar to that of a biological fluid- for an illicit injector it would be impossible. A large injection of solution with a lower osmotic pressure than blood (hypotonic) can lead to blood cells swelling rapidly and bursting. This is dangerous if a large number of cells are affected. In a hypertonic solution, the blood cells shrink and crenate, however, this is reversible and not as serious as the previous situation. As the solution travels along the vein, it is diluted and these effects are countered.

The pH of an injection is also of importance, as a very acid or alkaline solution may cause irritation or even tissue necrosis if extravasation occurs upon injection. For intravenous injections, this is of lower importance, especially if the injection is given slowly as the blood will buffer and dilute the solution quickly. The pH is more important for subcutaneous or intramuscular (or any other extravascular) injections, this can become very important and lead to further problems, for instance those outlines in the microbiological work introduction (section 5.1.2).

Thrombophlebitis and phlebitis are commonly reported in intravenous drug users and is anecdotally associated with the use of acidifiers, although no research has been conducted to confirm this.

The use of acids contained within mixtures, for instance from Vitamin C tablets or effervescent drinks, may result in complications caused by the injection of the additional extra materials.

1.11.2 Drug paraphernalia laws

Prior to the 1st of August 2003, Section 9a of the Misuse of Drugs Act (1971) in Great Britain made the supply of any articles known to be used for the administration or the preparation of an illicit drug unlawful. The only exemption from this rule was needles and syringes. Section 9a Misuse of Drugs Act made it illegal to supply, or offer to supply any article which the supplier believed may be

used, or adapted to be used, in the unlawful administration (including self administration) of drugs. Prosecution for the offence could result in a fine of up to £5000, a six month prison sentence, or both. Subsection 2 allowed the supply of needles and syringes to reduce the spread of HIV.

On the 1st of August 2003, the Misuse of Drugs (Amendment) (No.2) Regulations 2003 (2003b) came into force. This enabled the supply of items including swabs, utensils (spoons/'cookers'), filters, sterile water and citric acid by certain groups of healthcare staff.

The work within this thesis was conceived and the interviews conducted before August 2003. This work therefore describes the use of acids that were obtained 'illicitly' on the most part. The acids would have been obtained from food stores (lemon juice, fresh lemons, vinegar, citric acid) or pharmacies (citric acid powder, Vitamin C powder or tablets).

Some acids would have been obtained from needle exchanges during this time. Before the change of law, many needle exchanges had agreements with their local police force that ensured they would not be prosecuted for supplying acids to injectors, even though they were breaking the law through making the supply (Burton et al. 2003;Scott 2005a).

That change in the law did not, however, allow for the supply of other acids including ascorbic acid. It only states "citric acid". Citric acid appears to have been chosen for supply on the grounds that it is probably the most commonly used acid. There was no evidence present at the time of this change in the law on which to base this choice on the grounds of risk reduction, safety or suitability.

It wasn't until 2005 that the law was changed again to allow the supply of ascorbic acid, thus giving injectors who preferred to use ascorbic acid access to the pure powder form.

1.12 The use of acids in the injection preparation process- Previous investigations

As mentioned previously in section 1.6.1, many papers have mentioned the use of acids in the injection preparation process, but few have actually looked at the process itself, the acids used, how they are used and the risks

involved. Huizer (1987) along with others (Cook & Jeffcoat 1990) have studied the smoking of heroin in detail, producing information about the use of different salt forms, their absorption and metabolism. Illicit injections have not been studied in a similar manner.

The three main papers in the area are by Page (1999), Strang (2001) and Scott (2000). Another important document in this area was prepared by the Hungerford mobile needle exchange team in London (Wilkinson & Hungerford Mobile Needle Exchange 2002).

As described earlier, the paper by Page is concerned with the use of lemon juice to dissolve illicit heroin, as they appear to have believed they had discovered a new practice. The paper by Strang et al. was a response to this correcting the issues over the use of lemon juice by drug injectors, and providing insight into the use of acids in the UK.

The work by Scott (2000) is the only piece of work produced so far that explores the chemistry behind of the use of acid by injectors, however it does not actually look at harm in detail, and does no more than speculate upon possible risks. Using samples of street heroin in the lab, this work looked at the quantities of citric and ascorbic acids required to dissolve the maximum possible amount of diamorphine into the injection solution. The quantities of acids calculated in this work are an important factor that will inform the preparation of injections for use in this project.

The Hungerford needle exchange operates in the West End of London. In response to comments about the use of lemon juice by their clients, they decided to investigate the level of use.

They found that 28% (number of interviewees not stated in report) of their clients used citric or vitamin C all of the time, however mainly due to the poor availability of citric and ascorbic acids in the West End area, 68% of clients were using lemon juice "some of the time". This piece of work also looked at health problems that users had experienced from the acids they used- no other work has done this previously. The interviewees were asked if they had had experienced any problems with their eyes after injecting with lemon juice. 56% reported that they had experienced problems that included 'sore eyes', conjunctivitis, blindness, blurred vision, severe headaches and cataracts. Whether or not these problems are caused by lemon juice is open to question, but of the 44% who had not experienced problems, most were using acidifiers other than lemon juice.

1.12.1 Collecting data from drug injectors- The need for interviews

In order to prepare illicit drug injections in the laboratory in a representative manner to that of illicit users, it was imperative to learn their preparation methods. As stated, to date, there are no papers in the literature that outline the complete injection preparation process including details such as quantities used. Partial methods are outlined in a small number of papers (Scott, Winfield, Kennedy, & Bond 2000;Strang, Keaney, Butterworth, Noble, & Best 2001), along with anecdotal techniques outlined in various safer injecting guides/leaflets and user writings as previously discussed. None of these sources form a complete overview of preparation methods such that it could be reproduced in the laboratory setting.

Previous work, such as that conducted by Strang et al., The Caravan Project and Speed (Speed 1998;Strang, Keaney, Butterworth, Noble, & Best 2001), have recorded the acidifiers used by the injectors and the sources of water for the injection. These indicate the requirement of acid, or the use of different water sources, but they do not record the rest of the preparation procedure. Insight into the whole procedure might reveal further differences related to the use of these substances. For example, does the amount of water used to prepare an injection vary depending on the acidifier used, or does the heating time change, or are no changes made?

1.13 Research questions

The review of the literature, suggests that there are numerous aspects of the injection preparation process by illicit drug users for potential investigation. The following hypotheses were raised during the review of the literature:

Exactly how are injections produced?

The use of an acid appears common, and widespread amongst all crack and heroin injectors as suggested by previous literature; is this true? If so, what acids do injectors use? Do these acids pose risks?

What filters do injectors use? How effective are these filters, and do they themselves pose any risks?

How do illicit injections compare to pharmaceutically produced injections? What are the physical characteristics of an illicit injection? For instance, the pH, osmolality and particulate content.

What levels of drug are present within an illicit injection, and how does this vary through different preparation methods, for instance, with different acids?

Previous research has demonstrated the presence of viable micro-organisms within illicit heroin mixtures. There is a possibility these organisms remain viable after the preparation procedure and are found within the injection solution prior to injection. What are the microbiological risks posed by illicit injections?

Overall, it was aimed for the work to determine whether acid use in preparing injections could co-exist with safe injecting, or whether the use of any acid is so damaging that it would not be possible for harm reduction workers to recommend their use.

1.14 Project structure

In order to investigate the use of illicit drug injections, a two part project was devised.

The first section involved researching the methods current injectors use to prepare their injections. This required meeting with current injectors to learn about the preparation processes they use. This work gathered detailed descriptions of the entire process, as used by current injectors. These details were then used to form a standardised injection preparation formulation. This standard injection preparation process was used to prepare standardised injections within the laboratory.

The second section of work involved assays of these injections studying their contents, their microbiological risks and their physical properties. Results from these investigations enabled an assessment of the risk posed by these injections.

The following chapter describes the interview methods developed to investigate the processes used by drug users. The results produced by these interviews follow in chapter 3.

2 Interview design and methodology

2.1 Methodological considerations

To explore the methods of preparing injections used by current IDUs, two forms of investigation were considered potentially appropriate: focus groups and semi-structured interviews.

Focus groups, or group interviews involve an interviewer and multiple interviewees and the interviewees discuss the specific issue in question as a group. They would have provided data on the preparation processes and promoted the generation of qualitative discussion around methods and choices. The discussion of a focus group generates ideas and discusses key points surrounding an issue.

Focus groups have disadvantages. Such interviews would be unlikely to gather the preparation process of each individual in orderly detailed steps, possibly resulting in a random collection of data. This work aimed to record the spectrum of user techniques used by all participants. Focus groups can be dominated by particular individuals and all techniques may not be discussed due to the interactions between the particular individuals present in the group (Asch 1958). Focus groups can swing on the opinions of the members of the group.

Two of the main problems with the use of focus groups, especially with relation to this work, are their lack of direction and their difficulty with personal issues. This work required the investigation of an intricate procedure with the intention of its replication. Information had to be gathered during the one instance as re-interview was not possible. Therefore, control of the data flow by the investigator was critical to the collection of all the appropriate details. A focus group can lead to data getting confused, mixed up or omitted.

Injecting practices may be seen by some participants as a sensitive and personal topic. Some drug users may be embarrassed about their drug use. This was perceived in some of the interviews that were conducted. Due to this factor, some individuals may possibly have been unhappy to express their views and their technique (in what is an illegal procedure) in front of people with whom they may not know and feel uneasy. This factor could have been addressed by holding focus groups of friends, although arranging attendance at the focus

group at a set 'appointment time' could possibly have proved an issue. Recruitment of participants from a group of people present at a needle exchange at a single time point would negate the need to make prior arrangements, but could introduce privacy issues.

Consideration of these factors resulted in the decision to reject the use of focus groups.

One to one interviews conducted in a private room with just the interviewer and interviewee present were therefore the chosen method. This design would enable the interviewer to explore the required information from each person in depth, and enable the comparison of the same data collected from all of the other injectors interviewed.

2.2 Reliability and validity

An area of issue with any interviews, but especially within this area, is that of data reliability. This issue has been in debate in the research areas of criminality, drug use and alcoholism.

During this investigation it would have been possible that, for instance, the participants may have over estimated the amount of drug they would use. The answers given by the interviewees are not verifiable. It is not possible to triangulate the data with another source to confirm its reliability.

Maddux and Desmond (1975) found that chronic heroin users tend to give reliable and valid life history information when interviewed, although they do state that all information should be carefully judged.

By having face to face interviews, it was aimed to build up a degree of rapport between the interviewer and interviewee, which would possibly induce truthful responses.

2.3 Interview location

Potential interview locations would have to meet certain criteria to ensure they were suitable for interviewing.

Firstly, it would have to be a place injectors trusted and would feel safe to be interviewed. Secondly, it would be crucial to have a private room for the interviews.

Needle exchanges which were willing to allow interviewing were the most suitable location. It was considered that participants would feel comfortable in the surroundings and be willing to discuss their injecting there as they did it on a regular basis. It was important that the service would be able to offer a private room for the interviews.

During the interview planning stage other locations where current injectors were regularly found were considered. These included police stations, pharmacies, drug treatment clinics/doctors surgeries and hostels.

Police stations would have been completely inappropriate for these interviews as the participants would possibly be in a distressed state, and with the nature of the interview, it would make the participants consider the motives of the interview.

Pharmacies would provide access to injectors, but possibly in low numbers. The environment is appropriate, but the availability of private rooms was a critical factor- most pharmacies did not have such facilities at the time the work was planned.

Drug treatment clinics where injectors, or former injectors, attend would allow access to large numbers of potential participants. However, the perceived threat of sanctions from the clinic, such as withholding of substitute medication, may have deterred potential participants.

Hostels possibly might have been a suitable location, although the prosecutions that followed the discovery of illicit drug supply at the Wintercomfort hostel in Cambridgeshire (Rice & Thomas 2001) suggested that hostels may have been reluctant to be involved in a study of this nature.

2.4 Interview outcomes

The aims of the interviews were two-fold. Firstly, the interview would be used to collect demographic data. Secondly, the technique employed to prepare a drug for injection was to be investigated. The interview was therefore designed to meet these goals.

2.5 Methods

2.5.1 Interview locations

Three services that were approached agreed to interviews being conducted at their premises. These were:

BADAS	-	Bath Area Drugs Advisory Service
DASH	-	Drugs and Alcohol Service Hereford
BDP	-	Bristol Drugs Project

BADAS and BDP are voluntary sector agencies that receive NHS funding. DASH is a statutory (NHS) facility.

These services were approached for a number of reasons. Firstly was that of convenience and the ability to travel easily to them for interviews to be conducted; before interviews commenced it was unclear how long it would require the interviewer to be present within the service before a suitable number of participants had been interviewed. Frequent travel to distant places would raise costs and be impractical. These services were also approached on the basis that there were existing contacts with service managers, and they were interested in the nature of the work.

The interviews at Bath were conducted first, as 'pilot' interviews. This enabled going back to the University to implement any required changes, such as reprinting questionnaires to remove flaws.

2.6 Ethics committee approval

Diener and Crandall (1978) outlined four main areas where issues can arise within interview work :

Where harm occurs to the participants

Where there is lack of informed consent of the participants

Where there is invasion of privacy

Where deception or coercion is involved

For these reasons interview work is closely scrutinised to protect the participants and the researchers. Research involving human subjects should be conducted according to appropriate and accepted guidance in order to protect both the participant and the researcher.

In 1964, the "World Medical Association Declaration of Helsinki" originally outlined ethical principles for researchers in medical research. Since then, the document has been revised on a number of occasions to adapt to current medical practices. The declaration is the definitive code detailing ethical practice in health related research. Included within its principles are the freedom of the individual to participate or not, the need for consent to be informed and the risks to the individual be minimised and balanced against the need for the research and the potential outcomes. Within healthcare, these principles are assured by NHS research ethics committee assessment of work before it is conducted.

The nature of these interviews could have had negative effects upon the interviewees. For instance, the participants may have felt coerced into the work by the threat of repercussions for non participation, or where the work may have invaded their privacy. This work could possibly have exposed the vulnerabilities of the participants.

Research ethics committees assess the safety, appropriateness and quality of proposed research in the context of adherence to ethical principles. Prior to any research work commencing in a region an application needs to be made to the appropriate regional committee. For each of the three interview locations for these interviews, applications were made to the Local Research Ethics Committee (LREC).

The interviews, including the questionnaire and demonstration were carefully designed to prevent avoid any problems in the four areas (above) arising.

The initial application to the Bath LREC included the use consent forms to be initialled by the interviewees prior to interview commencement, and payment for their time in the form of shop vouchers to the value of ten pounds. The committee felt that the use of consent forms would have no legal bearing as they were only initialled to prevent anonymity, and therefore there was little point in it. Consent was obtained from the participants verbally after they had been informed of the nature of the study and what the interview entailed. Additionally, the committee felt the payment of volunteers was coercive. The payment of volunteers was not adopted during the Bath interviews, however it was reintroduced to the applications for the Hereford and Bristol interviews, after services requested it and accepted by those LRECs. The payment of volunteers was made to compensate them for their time.

In addition to the LREC application, before work could be conducted in Hereford, an application had to be made to the local NHS Research Governance Committee as the agency in which the work was proposed is an NHS service. This is a committee that reviews all work carried out in Trust facilities to ensure NHS resources are not used to support research without reimbursement.

The letters of ethical approval from the LRECs and the approval letter from Hereford NHS Research Governance Committee can be found in Appendix 1.

2.7 Interview design

2.7.1 Section One – Semi-structured questionnaire

To investigate the participant's background, it was decided to produce a semi-structured interview, asking a mixture of closed and open questions. This formed the first section of the interview process.

A copy of the list of questions posed to the participants is located in Appendix 2. It includes questions exploring the interviewee's demographics, their drug history, their injecting history and then focuses on their use of acids in

their injection preparation method. The questionnaire consisted of open questions, with lists of probes that were read out to the injectors, such as in question 20. The probes were included to remind the interviewees of acids that are occasionally used that they may have used, but forgotten to declare in the first part of the question.

In summary, the interview collected data including age, gender, current main drug of use, length of time injecting, in which town/city/locality they first learned to prepare injections, the injection site they currently use, and to list all the drugs they have ever injected. The questions then focussed primarily upon acid use. The participants were asked what acid they preferred to use, which they usually used, where they obtained the acids from, what they had ever used, and if they had ever had any health problems that they attributed to the use of an acid.

Responses to the questionnaire section of the interview would be used to investigate any unexpected results in the second stage of the interview. For example, the preparation technique of an older injector may have been found to be very different to that of younger injectors. Similarly, an interview with an injector who had learned to inject a drug in a different country may have produced similar results. The recording of the participant's background would highlight any issues such as these, and then any outlying results disregarded if necessary.

2.7.2 Section Two- Preparation investigation

To prepare injections in an identical manner to that of current injectors, detailed information would be required concerning the process, for instance quantities and procedural steps. This information would be collected from current injectors, and for this purpose, four main techniques were considered:

i. Injectors could be asked the questions regarding their preparation process
ii. The injectors could be asked to describe the process from memory
iii. The injectors could be asked about the process (as in either 1. or 2.) with some quantitative input, for instance using powder to demonstrate quantities of substances used

iv. They could be observed preparing an injection with quantifiable data measured and recorded.

Investigation of the process through a series of questions posed by the interviewer was the first considered option of detailing the injection preparation process. Using a prepared list of questions, at each step of the process the participants would be asked what equipment they would use, how they use it, how much they would use and so forth.

This method would be very easy to accomplish, and require minimal consideration of the entire process for the participants other than to answer to the current question, for example: Q: 'What water do you use?'
A: 'Water from a tap.'
The progression of the process would provide structure to the questioning.

The main problem with this approach was that it would assume that all injectors prepare injections in a similar sequence, that is, the order of the questions being asked would presume the order of the sequence.

The level of detail provided by each participant would be likely to vary as well. Using the previous example, one user might state that they acquire their water from 'a tap', whereas others might state that they get it from the bathroom tap, or the kitchen tap. A participant volunteering more information might reveal that they get it from a tap, but they pour it into a cup which stays on the lounge table until it needs refilling.

Additionally, this type of questioning risks stimulating very little thought into the way they perform the complete procedure. This could possibly result in actions that they usually performed not being recalled if it was not asked about. Other than giving the required answer to the current question they might not state important procedural steps, for instance, the wiping down of a spoon with an alcohol swab before use. The difficulty in recalling the entire technique in detail may result in the description being disjointed. Recall bias such as this was of concern in the selection of the interview procedure.

Another flaw in this technique is the lack of quantitative input from the interviewees. At best, users would be able to verbally describe how much they use e.g. 'a pinch of acid', but this is vague and will vary from pinch to pinch, and from person to person. This data was a critical requirement of these interviews in order to translate it into a laboratory process.

The second method considered involved less structured interviews, using a more open questioning style asking the participants to talk through a description of their usual way of preparing an injection. This technique has the advantage of allowing in depth detail descriptions of the technique, as well as not assuming any particular procedural order. This method has the advantage of being unlikely to 'lead' the participants and may result in a more thorough explanation of all actions performed.

This method would suffer from the same problems associated with recall as the direct questioning method, above, possibly even more so as the participants would be given no prompting [by the questions]. Additionally, it would also provide no quantitative data regarding the materials used during the preparation.

In order to obtain quantitative data in interviews, previous researchers in this field have provided the interview participants with substances such as salt. The participants were then asked to measure out a quantity similar to that of the acid that they used. The researchers however, had assumed that the weight of salt used was equal to the weight of acid used by the injectors. Having different molecular weights and densities this is of course not the case, although equivalents could be matched through volume comparisons (this work was conducted during the evaluation of the supply of citric acid to injectors in Glasgow, although it was not included in the final report (Garden et al. 2003)).

Even though, this was a flaw within the work that employed it, it demonstrated that the technique was a viable means of obtaining quantity measurements in interviews. The possibility of utilising a similar technique incorporated into the above interview designs was explored.

2.7.3 Observation of injection preparation in naturalistic environment

The observation and recording of the preparation of actual drug injections by injectors within their usual naturalistic injecting environment was an option considered but rejected. This method would have enabled the recoding of the procedure with accuracy and detail, limited by the data recording skills of the researcher. This type of study is referred to as non-participant observation.

The major issue with this type of study was with the ethics. The observer would be placed into potentially risky situations observing illegal activity. Alongside this, there was the risk that they might be present at a police arrest, or witness to an overdose. Both of these events would put researchers in a precarious position. At the time of interview planning, work of this nature had not been previously conducted. In consideration of these points, the possibility of acquiring ethics approval by a PhD student for such work was considered low, based on previous discussions with ethics committees. Therefore, the design of an interview incorporating such observation was not pursued.

Additionally, and very importantly with regard to this work, there would be no opportunity for the measurement of quantities of materials used. This would have a large impact on the usefulness of the data in the derivation of the laboratory injection preparation procedure.

2.7.4 Interview with demonstration of preparation

After consideration, the idea of a practical demonstration of injection preparation, in the needle exchange environment, was devised.

The primary advantage of using this procedure was obtaining quantitative data (for the materials used including water, acid and drug quantities, along with heating time), and the ability to observe and record all aspects of the procedure. This would include points that interviewees might not recall in an interview. Secondly, by following their usual preparation procedure, the participants' recall of their procedure would be prompted by the process.

It was envisaged that the inclusion of the practical element of the interview would make the interview more appealing and interesting to potential interviewees and thus improve recruitment.

There were three main disadvantages envisaged in this work. Firstly, these interviews would require setting up time beforehand, in addition to the usual of questionnaire printing, equipment and materials would need to collected and organised. Secondly, the time to prepare an injection could possibly have extended the length of the interview beyond an acceptable level.

Most critically, there was the problem of the drug. Using Class A drugs in an interview would pose many implications. Legally, there was the possibility of theft or robbery of samples, plus the supply of the drug to the participant for

demonstration would constitute an illegal supply. The possibility that a participant may attempt to ingest either the drug or the finished injection solution would pose potential problems, and pharmacologically there was risk to both the participant and interviewer from exposure to drug material.

The use of real drug within interview circumstances was not an option. In order to perform the preparation, inert substances would have to be used in place of real drug material.

2.7.5 Imitation drug substances

In order to prepare the injections as realistically as possible, substances with similar properties as illicit heroin or crack had to be identified. These substances needed to have similar physio-chemical properties as the drugs, with regard to water and acid solution solubility. Additionally, the substances chosen had to have little, or no, pharmacological activity, be acceptably innocuous and inexpensive. The chemical chosen had to be safe for use under the circumstances, but also if it got stolen, then consumed, it had to be non-toxic.

Biologically inactive opiate compounds were explored for use as the 'drug' during the interview, because their chemical properties are very similar to that of heroin, however, their legal status prevented serious consideration. Thebaine is an ideal substitute physio-chemically, but it is a schedule two controlled drug- the same as heroin- even though it is pharmacologically inactive.

The selection of a suitable compound proved difficult. In the Glasgow area, it is common to find heated and crushed paracetamol tablet powder on the illicit drug market as a heroin diluent (Personal Communication, Roberts, 2002). Preparing paracetamol tablets in this manner produces a substance visually similar to illicit heroin.

Paracetamol is also one of the most commonly used diluents in illicit heroin base samples (King 1997), and for that it was considered likely to confer some of its physio-chemical properties to that of the illicit heroin powder. At the outset of the demonstration, the participants were informed of the fake 'drug' material not being the real drug. Some of the participants were inquisitive enough to ask what the powder they were using was, a few almost seeming to

believe it actually *was* real drug, its appearance being so similar. Once they were informed of its composition, interest in the powder waned. The nature of paracetamol and its commonly known effects proved a disincentive if the interviewees enquired about the powder.

Heroin substitute manufacture

This was produced from paracetamol tablets manufactured by Sterwin (Guildford, Surrey).

Each tablet contained:

Paracetamol	500mg
Soluble Starch	50mg
Maize Starch	21.4mg
Purified Talc	15mg
Stearic Acid	5mg
Polyvinyl Povidone Pyrrolidone	2mg
Potassium Sorbate	0.6mg

The first step of the process was to crush the tablets down into powder using a mortar and pestle. Each tablet would produce approximately 500mg of finished fake drug powder. This powder was then placed on a hotplate. On heating, the powder melted to form a liquid, which on continued heating turned dark brown in colour. The solution was then immediately poured back into the mortar and allowed to cool and solidify. Once the liquid had thoroughly solidified, it was re-crushed into a powder. This powder was brown in colour and was the finished heroin substitute.

Testing of heroin substitute

The powder produced was measured using melting point apparatus and found to be almost the same as that of pure paracetamol (167°C compared to 169°C of pure paracetamol).

As a test more appropriate to the interviews, the powder was tested in the injection preparation situation. Using 100mg of powder, and 50mg of citric acid with 1ml of water, the powder dissolved into solution and was able to be drawn up through a Swan filter into the syringe. The end result was a dark brown solution in the syringe. The powder had dissolved completely into solution

without leaving residue in the spoon. The powder was considered suitable as the substitute.

The crack substitute

The identification of material to form the crack substitute was more difficult than that of the heroin powder. Crack cocaine is almost 100% cocaine base- approximately 90% cocaine base, with 10% or less sodium bicarbonate (Jones 1990). It may also contain various impurities (that were present in the cocaine hydrochloride powder from which it was produced) that have been incorporated within it during the manufacturing process. These can include lidocaine and procaine (Griffin 2003).

Identification of a compound to simulate crack was even more difficult than for heroin. Attempts were made to synthesise fake crack 'rocks' that resembled those of real crack- these were required to have a similar water solubility.

In order to keep the compounds in use simple and safe, the decision was made to synthesise the crack from a paracetamol based mixture. Paracetamol powder was mixed with sodium bicarbonate powder in water, but this failed to produce solid 'rocks'. The use of paracetamol tablets as the source of paracetamol was subsequently attempted. Starting with crushed paracetamol tablet powder, sodium bicarbonate was added to make up 10% of the total weight. Nine millilitres of water was then added per ten grams of powder to form a paste. The paste was then dried forming a block that could be broken into 'rocks' which were hard and did not crumble. These were suitable for the interviews.

This fake crack would pose little risk to participants – no more than that posed by a single paracetamol tablet. This was essential in the consideration of the required Ethics committee applications in order to prove safety.

2.7.6 Materials use during interview

For use in the interviews, approximately 500mg of fake heroin powder was measured out into eppendorf tubes and the weight accurately recorded. One rock of fake crack was placed in each tube and the weight accurately recorded. Citric and ascorbic acids were also measured out in this manner, with

500mg per tube. For each interview, there was a set of all four tubes available. Depending on the injection prepared, either one of the drug tubes would be used, or both for a speedball preparation. Also one of the acid tubes would be used, depending on the choice. If lemon juice or vinegar was chosen, the volume used would be recorded during the interview.

After the interviews, the contents of the tubes were reweighed, and the difference calculated to establish the exact quantity of materials used by each participant.

2.7.7 Demonstration Equipment

To prepare injections equipment such as lighters, spoons and filters are required. It was chosen to supply all equipment that may have been required during the process. By supplying all the equipment, there would be no variation of the results due to use of different equipment, for instance spoons. A bigger or thicker metal spoon would possibly require a longer heating time compared to a smaller or thinner spoon. Additionally, the metal alloy would also have such an effect.

The equipment was kept out of sight from the participant in a box. To get an item for use, they had to request it from the interviewer. The process of this blinding was to necessitate the user to consider the equipment they use. Leaving the equipment on a table in full view, would not have the same effect in stimulating the user to think about the item they required. For instance, if there was a dessert spoon and a tea spoon on the table they may pick up the nearest out of convenience, even though they normally used the other type.

2.8 Craving inducement

Studies on the craving of drugs illustrate that this could potentially be induced in the participants during the interview work. This would be of significant concern. The potential to induce craving within the participant of the interviews was of concern, and it would be considered unethical if steps were not taken to forewarn and protect the participants of this risk. It was considered that craving

could result in ex-injectors being encouraged to return to injecting, or encouraging current injectors to inject additional drug doses.

To prevent this occurring, a number of steps were taken. Firstly, exclusion criteria during recruitment would ensure ex-injectors would not be interviewed. This would prevent the risk of encouraging them to re-pursue injecting, and additionally would also ensure that observed preparations were current techniques in use, not ones that might be aged, or affected by poor memory recall.

When interviewing, before the interview began, and then again before the demonstration was started, all participants were warned that craving could possibly be induced by the process of preparing an injection. They were asked if they thought this would be an issue for them if it occurred. If they said they thought it might, or if the interviewer had specific concerns, the interview could proceed as a series of questions without the demonstration. The list of questions drawn up for these circumstances is found in Appendix 3.

2.9 Data recording

The questions were asked by the interviewer, who wrote down the responses. The responses were transcribed verbatim, unless the response was long. Long responses had the key points written verbatim. Each questionnaire document was marked with an interview site code and an interview number to enable identification, and ensure the anonymity of the participant.

2.9.1 Demonstration recording

The method of recording the demonstration techniques posed challenges. The chosen method had to be ethically and technically feasible, whilst producing suitable data for analysis.

Four main methods were considered:

- Interviewer observation with note taking
- Audio tape recording of process narrated by participant or researcher, with observations noted
- Still photography of each significant step

- Video tape recording of entire process

The most appropriate method considered was video recording. This method would provide a permanent record of the techniques used, with the ability to be reviewed on numerous occasions. However, it would not provide much data with regards materials used. Amounts would be visible on the recording, but not quantifiable.

The confidentiality of video recordings was of additional concern. The videos could enable the identification of a person preparing an injection, this would breach confidentiality. Even careful camera placement to record only the hands could still reveal clues as to the identity of the participant if they had identifying features such as scars, tattoos or jewellery. Discussion with members of the Research Ethics Committee for the Bath area confirmed this would be problematic in obtaining ethical approval.

The final potential anticipated problem with the use of video recording would be the effect of the camera on the participants. This could occur in two different ways. Firstly, the participant might be anxious of the camera. This would possibly induce errors in their preparation process, may have caused nervousness and tremor leading to preparation difficulties, or they might rush to get it over with. In a similar manner, interviewees might become nervous due the prospect of being recorded performing the procedure, either on the grounds of legal implications, or embarrassment of demonstrating a part of their private life of which they are ashamed. Alternatively, it is possible that the participant might 'play up' for the camera, and proceed through the preparation in an unnatural way.

In light of these points, although considered methodologically the best means, the use of video recording was rejected as a means of gathering the interview data on ethical and practical grounds.

A set of still photographs would lack data regarding what occurred during the times between the photographs, in order to overcome this, a large number of photographs would be required, and the preparation process might be unduly delayed by the stops for the photographs to be taken. Still photography with a photographic camera was considered, but the confidentiality of the interviewee would still potentially be breached through possible identification from the photographs. Use of this method would have been a poor substitute for video recording (which is continual and has sound) and would have possibly have missed vital information, so the method was rejected.

Audio recording would have been beneficial for the questionnaire section of the interview, but would have been of questionable value for the demonstration section. The demonstration would still have required observation recording using an additional method. Audio tapes would have required transcription after the interviews were conducted in addition to the observational reports analysis.

2.9.2 Interviewer observation and recording

Interviewer observation would require watching the process and documenting it as it happened.

After consideration and rejection of the alternative methods mentioned above, this method was investigated. The method required no electronic recording equipment, but would produce a record of all points observed and recorded by the observer during the preparation process. There was also the option of recording comments made by the participants as they proceeded. The simplicity of the technique was attractive, although it was unclear if it was feasible in practice.

Trials of this technique demonstrated that this was a difficult task. It required rapid note taking with care to ensure all important details were not missed. Simply writing down every point as it occurred proved impossible.

In order to speed up the recording process, checklists were produced that made it easy to record actions. For example, by ticking a box, rather than having to write long-hand 'dessert spoon' to record this selection.

2.10 Recruitment

Recruitment of participants was a major factor in determining the success of the interviews. The availability and willingness of people to be interviewed was critical to the outcome of this work. Lack of potential interviewees would increase the overall time taken for this work to be completed,

and could reduce the final amount of data collected. Careful consideration was therefore given to this matter.

To encourage potential interviewees to participate, the interview was designed to be completed in a relatively short time (approximately 15 minutes) thus inconveniencing people as little as possible.

Injectors approached to participate may have been suspicious of the motives of the interview and/or the reasons for the interview (Matheson 1998). It could possibly have been viewed as an undercover police operation to arrest drug users. To prevent this, all users were reassured about the confidentiality of the interviews. Additionally, before an interview took place, they were explained its nature and its purpose in detail. Finally, it was hoped that by conducting the interviews at their needle exchange the users would feel some degree of trust and security.

Participants were informed at the beginning of every interview that they could leave at any point, without needing to give a reason and that if this it occurred would not affect their treatment at the needle exchange in any way.

Leaflets and posters were used to promote the research to service users and displayed in waiting rooms and needle exchange areas within the participating agencies. Exchange users were also informed of the research during the exchange process. A copy of the leaflet used is found in Appendix 4.

Needle exchange staff were given briefing talks on the nature of the work to inform them of the nature and aims of the work being conducted. This in turn enabled them to inform users about the work to aid recruitment.

The recruitment process began two weeks before the interviews. All volunteers were given at least twenty-four hours to consider participation before consent was obtained.

To ensure appropriate participants were recruited, three inclusion criteria were placed upon the volunteers. Firstly, as discussed previously, no ex-injectors were interviewed, all participants had to be currently injecting. Secondly, all participants had to be over 18 years old.

Thirdly, the person must have injected for a minimum of 3 months prior to interview. The reasoning behind this was two-fold, and aimed to protect both the participant as well as the data. A new injector could possibly have been exposed to potentially dangerous information, for instance, the acidifier probes (especially descalers) would highlight the ability of using these substances. A

person who has injected for less than three months was considered to possibly have less experience than desired to inform the research about an established working process. They may skew results through the use of inaccurate procedures or quantities.

Finally, it was important that the person also actually prepared their injections themselves. The preparation of their injections could be done by a partner or friend. A demonstration of preparation by someone who had only observed the process would be likely to include errors or omissions. Volunteers were asked whether they produced their own injections before recruitment.

If a volunteer met these criteria, they were suitable for participation.

2.10.1 Sample size selection

The total numbers of participants interviewed was based on the qualitative research basis of data saturation. As the interviews were conducted, the data being recorded, especially with regard to the demonstrations, was monitored. Once the interviewer was seeing or hearing no new information, data saturation had been achieved. Further interviewing will only provide excessive data, and new results are the exceptions rather than typical.

2.11 The interview process

The interviews were conducted in a private room at each of the exchange facilities. Privacy was critical for confidentiality. It would also avoid intrusions, interference from other people that could contaminate results and the sharing of 'bad' practices by demonstration of them to other IDUs. The privacy would also offer a level of reassurance to the participants. The process of injection preparation is an illicit and illegal practice that the participants may have been worried about observation by non-participants, either other injectors or service staff. Some participants may have been embarrassed conducting the procedure and be reluctant for the process to be witnessed by anyone other than the interviewer.

The interviews were designed to last approximately 15 minutes. The actual length of the interview would depend on how much detail the respondents

went into, and how long their injection preparation took. In a three hour interviewing session, ten interviews were estimated as possible. The attention required from the researcher observing and recording the procedure also limited the maximum number, as mental fatigue could impact on recording quality.

At the outset of each interview, the participant was told what would happen and the reason the research was being conducted. It appeared that many of the volunteers agreed with the cause of the work and understood there was potential benefit for all injectors. All volunteers were reminded that they could leave the interview at any time without needing to give a reason and doing so would not affect their service from the needle exchange. If the volunteer was satisfied, the interview commenced.

After the interview concluded the participant was thanked for their time and input. They were then offered the ten pound gift voucher (during Hereford and Bristol interviews). After the participant had left the room, the fake drug and acid powder containing tubes were collected in numbered and coded bags, along with the questionnaire and observation checklist. Any additional materials of importance were collected such as filters. Used syringes were disposed of in sharps bins- there was no risk during this as it had been observed that they had not been used for injecting, if there had been (for instance an accidental prick with the needle) the participant would have been requested to dispose of it themselves in the provided bin. The room was then made ready for the next interview.

3 Interview results

3.1.1 Interview piloting and problems encountered

The pilot interviews in Bath highlighted problems with the demonstration section of the process, and introduced a problem that was unanticipated.

The interviews in Bath were discussed with BADAS during the submission phase of the ethics application. The protocol for work was agreed and dates set for the interviews once approval had been obtained. On the first day of interviewing, it became apparent that the contact worker at the service was under the impression that the interviews would take place with him present. The presence of a member of staff in the interview room would breach the confidentiality of the participant, and was considered by the research team to be unethical.

The member of staff was explained the situation, but stated that after consultation with his director, it was agreed that he was required during the interviews for health and safety reasons.

This situation was not expected and it was felt that the presence of this worker would adversely affect the responses given by the participants. Additionally, his presence was not agreed with the ethics committee during the application for work, so it was referred back to them for consultation. A new information sheet was drafted for potential participants that stated that there would be a member of needle exchange staff present during the interviews. The LREC decided that the presence of a member of staff, combined with the new information sheet was acceptable.

The first design of the checklist for use during the interviews consisted of a double page section for each envisaged step of the process. In practice, flaws in this design became evident rapidly. The large number of pages and sections, combined with the speed of the preparation process by the participants led to difficulty in keeping up with the recording of the process, and in the event, details not being recorded. To correct this, the checklist was redesigned to enable all the text to fit on the two sides of A4 paper. This revised final version can be found in appendix 5.

3.1.2 Interview completion

The interviews were conducted over a total of eleven days, however, these days were not consecutive, but spaced out over a period of four months due to location changes and delays for ethics committee site specific approval (Hereford and Bristol).

3.1.3 Interview analysis

Firstly, the used demonstration materials were accurately weighed and the amounts used calculated.

To group data with the same theme, codes were assigned for each question and part of the demonstration, but the data for the questionnaire was kept separate from the data from that of the demonstration checklist. Both sets of data were identifiable by interview number and location if cross-referencing was required between the question data and the demonstration data. The separation of the data was conducted to make the data files more manageable and smaller in size.

This data was then entered into a Microsoft® Access database (Part of Office XP Suite. Redmond, Washington, U.S.) for storage and analysis.

Themes and trends from within the results were then grouped and explored. Data was further analysed by input into SPSS (Statistical Package for Social Scientists, Versions 10 and 11. SPSS Inc. Chicago, Illinois, U.S.).

3.2 Results

In total, sixty five injectors were interviewed. Two of the participants were from BADAS (the pilots), twenty-eight from DASH, and thirty-five from BDP. The results from the pilot interviews were included in the final results as the changes made to the interviews after piloting were very minor (the checklist change) and most importantly, it was clear that the results from the pilots were highly similar to those obtained in the main interviews.

3.3 Part 1 - Interview results

3.3.1 Demographics

Eighty percent (n=52) of participants were male, 20% (n=13) were female (ratio 4:1). The mean age of all participants was 28.4 years. The mean age of the male participants was 29.1 years (range: 20 to 46 years), of the females it was 25.8 years (range: 18 to 38 years). 34% (n=22) of the participants were under 25 years old, 66% (n=43) were 25 years old or older. Table 3 shows the age and gender of the participants per location.

Table 3. Gender and ages of participants by location

Location	Gender	Number	Mean age and range
Bath	Male	2	30.5 (26 & 36)
Hereford	Male	22	26.9 (range 20 to 37 years).
	Female	6	23.8 (range 18 to 36 years)
Bristol	Male	28	30.7 (range 20 to 46 years)
	Female	7	27.6 (range 19 to 38 years)

3.3.2 Drug use

Table 4 presents the main drug of use by the interview participants at each location.

Table 4. Main drug of use by participants by location

Location	Drug	Number
Bath	Heroin	1
	Speedball	1
Hereford	Heroin	28
Bristol	Heroin	18
	Crack	3
	Speedball	14

Drug use by gender

All female participants only injected heroin. The crack and speedball injectors interviewed comprised solely of male participants.

Drug use by age:

Table 5 presents the age of the participants in relation to the main drug of use

Table 5. Age of drug users in relation to the main drug of use

Drug	Mean age of users	Age Range
Heroin	31.6 years	19 to 46 years (SD 7.18)
Crack	33.7 years	24 to 42 years (SD 9.07)
Speedball	27.4 years	20 to 39 years (SD 5.73)

3.3.3 Length of time injected

Table 6 presents the time participants have injected for at each location.

Table 6 Length of time participants at each location have injected

Location	Mean time injected
Bath	8.5 years (range 7 to 10 years, SD 2.1)
Hereford	5.7 years (range 3 months to 18 years, SD 4.3)
Bristol	6.8 years (range 3 months to 15 years, SD 4.2)

3.3.4 Drug history

Location of first drug injection

This was separated into local to the interview location, or distant.

Bath

One participant first injected in Bath, one did not.

Hereford

Five of the 28 participants first injected somewhere other than Hereford.

Bristol

Sixteen of the 35 participants were not originally from Bristol and had injected for the first time elsewhere.

3.3.5 Drugs ever injected

Participants were asked to list all the drugs they had ever injected, even just once. These are listed in Table 7.
(The results for Bristol and Bath have been amalgamated on the basis that they are closely located which would potentially lead to similar drug sources, and because the number of Bath interviews (n=2) were low, there were no trends.)

Table 7. Drugs ever injected divided by region

Hereford

Drug	Number used	%
Heroin	28	100%
Amphetamine	19	68%
Crack	11	39%
'Speedball'	10	36%
MDMA (Ecstasy)	8	29%
Cocaine	8	29%
Diazepam	4	14%
Diconal[1]	3	11%
LSD	4	14%
Temazepam	3	11%
Morphine ampoules	3	11%
Palfium[2]	2	7%
Cyclizine	1	3

Bath and Bristol

Drug	Number used	%
Heroin	37	100%
Crack	34	92%
'Speedball' *	29	78%
Amphetamine	27	73%
Cocaine	16	46%
Temazepam (inc 'eggs')	8	22%
Diazepam (inc valium amps)	6	17%
Diconal[1]	7	19%
Palfium[2]	6	17%
MDMA (Ecstasy)	5	14%
Dihydrocodeine	4	11%
Morphine (inc. MST tablets)	3	9%
Cyclizine	3	9%
Temgesic	3	9%
Omnopon[3]	2	6%
Codeine	2	6%
LSD	1	3%
'Benzos' (unspecified)	1	3%
DMT	1	3%
Zimovane[4]	1	3%
Lorazepam amps	1	3%
Mandrax[5]	1	3%
'Black Bombers'[6]	1	3%

[1]Proprietary tablet formulation containing the opioid analgesic dipipanone and the anti-emetic cyclizine

[2]Proprietary tablet formulation of opioid analgesic dextromoramide

[3]Proprietary injection formulation of papaveretum (opiate analgesic)

[4]Proprietary tablet formulation of the hypnotic drug zopiclone

[5]Proprietary tablet formulation of the hypnotic drug methaqualone

[6]Slang term referring to defunct tablet formulation of amphetamine tablets

3.3.6 How the participants learned to prepare injections

All participants stated that they had learned to prepare injections by being in the presence of other injectors i.e. by watching them. These ranged from friends, partners and family members including parents.

Most participants stated that they had: 'watched others do it', or they 'picked it up from the crowd'.

One participant stated that he had learned the technique by 'watching his parents prepare injections as he was brought up'.

3.3.7 Materials used to prepare first injection

The participants were asked about the preparation of their first injection, and how they gauged the quantities of materials, particularly acid in this preparation.

The most common response to this question, by twenty-six participants (40%), was that they had a 'rough idea' in their heads of how much of each material was required.

Twenty (31%) of the participants interviewed described the measurement of the quantities as 'trial and error'. In other words, they guessed the amounts required.

Four participants (6%) stated that they had a 'good idea', or were confident that they knew how much of each component material was required for an injection to be prepared. This would assume they were alone or otherwise not directed during the conduction of the procedure.

Fourteen (22%) participants were actually shown by another injector, or told how much of each component to add.

No answer was recorded for one participant.

3.3.8 The use of acids

The participants were asked if they used an 'acid' in the process they followed to prepare injections. All 65 replied that they did.

Reasons given for acid use

The participants were then asked to explain why they used an acid in the process. The most common reason for using an acid revolved around the idea that the powder had to be broken down in some way. The most common response was:

'to break it down'

Similar replies included:

'To breakdown the heroin/gear/heroin compounds'

'Nothing happens. Gear doesn't break down'

'Citric cuts it down to liquid'

'Because it is dirty and you need to break it down'

'Breaks down what it is cut with'

'Break it down 'cos it's got loads of shit in it'

'Break it down and kill any germs'

These responses were made by 53 (81.5%) of the participants.

The concept that additional materials are present in drug material was expressed in the second set of reasons for using acid:

'Because of the rubbish and waxy stuff in it'

'Because of the crap in street heroin'

'To clean up the shit in heroin'

One participant answered that it was to 'turn it into gear' suggesting they thought the acid was required to somehow form the drug prior to injection.

One participant stated they used acid because they had been told to. Three participants stated that they did not know why they needed to use it. These answers suggest that none of these users understand or had any idea why they are using it, and that they only use it because they were shown that they need to.

Out of all of the participants, only four gave suggestions that described the real purpose in detail.

One suggested that as 'it is an alkaloid, it needs acid to make it go into solution'. Two used the word *dissolve* in their explanation:

'To help it dissolve'

'To dissolve the gear'

One participant appeared to have come to the same conclusions as Strang et al.(Strang, Keaney, Butterworth, Noble, & Best 2001) and stated:

'Brown heroin needs acid to dissolve in water' as opposed to white heroin.

3.3.9 Acids in use

To explore the use of acids by the sample, the participants were asked 'Which acidifier do you *prefer* to use?' and then, 'Which acid do you *normally* use?'.

It is important to note that the responses have to be split by interview location. This is due to the fact that the three exchanges used for the interviewing supplied either: citric and ascorbic acid (Hereford), citric acid only (Bath), or no acid at all (Bristol). At the time of interviewing, the Bristol exchange was unable to (and had never in the past) supplied acids to its users due to financial restrictions. Table 8 records the participant's responses.

Table 8. Acids preferred and normally used by injectors at each interview location.

Location	Preferred Acid Acid	n (%)	Acid normally used Acid	n (%)
Hereford	Citric	16 (57)	Citric	21 (75)
	Ascorbic	12 (43)	Ascorbic	5 (18)
			Citric or Ascorbic (No Preference)	1 (3.5)
			'Whatever is available'	1 (3.5)
Bristol	Citric	22 (63)	Citric	33 (94)
	Ascorbic	13 (37)	Citric or lemon Juice (Use each equally)	2 (6)
Bath	Ascorbic	2 (100)	Citric	2 (100)

3.3.10 Acidifiers ever used

Anecdotal reports suggest that drug injectors use a wide variety of substances in order to increase the solubility of illicit drugs. In order to investigate this, the participants were asked what they had *ever* used during injection preparation. They were asked what they had ever used, then a list of commonly used acids was read out as a prompt. The results are shown in Table 9.

Table 9. Substances ever used by injectors interviewed when attempting to dissolve illicit drugs.

Acidifier	Number of participants ever used (%)
Citric	65 (100)
Lemon Juice	60 (93)
Ascorbic acid/Vitamin C	53 (82)
Vinegar	44 (68)
Fresh Lemon	39 (55)
Descaler (any sort)	10 (15)
Fizzy sweet coatings	2 (3)
Vitamin C tablets	2 (3)
Orange Juice	2 (3)
Tartaric acid	2 (3)
Lime Juice	2 (3)
'Hot Water'	1 (2)
Polo Mint	1 (2)
Alcohol	1 (2)
Grapefruit	1 (2)
Bleach	1 (2)

3.4 Part 2 – Demonstration results and observations

The information collected during this work can be divided into two main categories- preparation steps, and details about the equipment and materials used to conduct these steps. The preparation steps are the major factors regarding the procedure; the materials and equipment details are considered the finer details.

3.4.1 Preparation steps

These are the overall order in which the participant prepared the injection. They are concerned solely with the major events within the preparation procedure. The overall procedure will require certain critical events to occur, without these events the preparation would not achieve the goal of preparing a viable injection solution.

Using anecdotal details available, the steps that form the injection procedure were used to devise the demonstration checklist. Although the anecdotal data informed about the steps used during preparation, they did not make the order of the steps clear, and variations were seen between the reports.

3.4.2 *Heroin injection preparation steps*

Forty-five of the forty-seven heroin injectors (96%) followed the same preparation steps to produce their injection. These are outlined below:

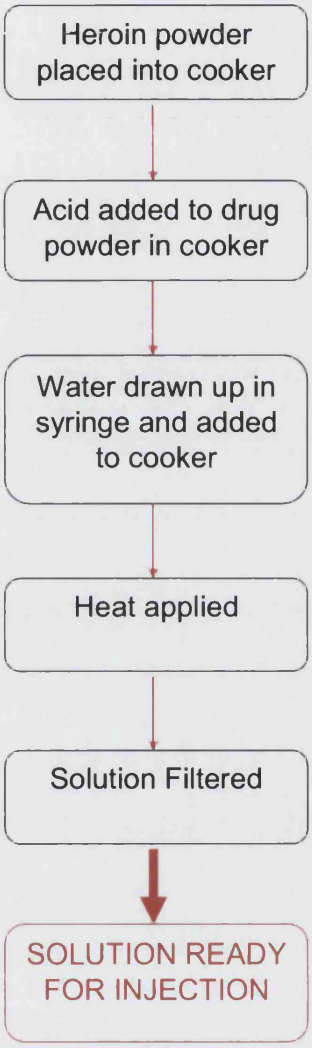


Figure 2. Steps used to prepare heroin injections by 96% of interview participants

Figure 2 shows the drug being mixed with the acid first, and then the addition of the water. The two participants who did not follow these steps demonstrated variation in the order they mixed the drug, the acid and the water. One of these added the water to the drug powder, before adding the acid powder. The second choose to measure out the acid powder, and then add the drug powder to it.

3.4.3 Crack injection preparation

Six of the eight participants (75%) who prepared crack in the demonstration followed the series of steps depicted below in Figure 3:

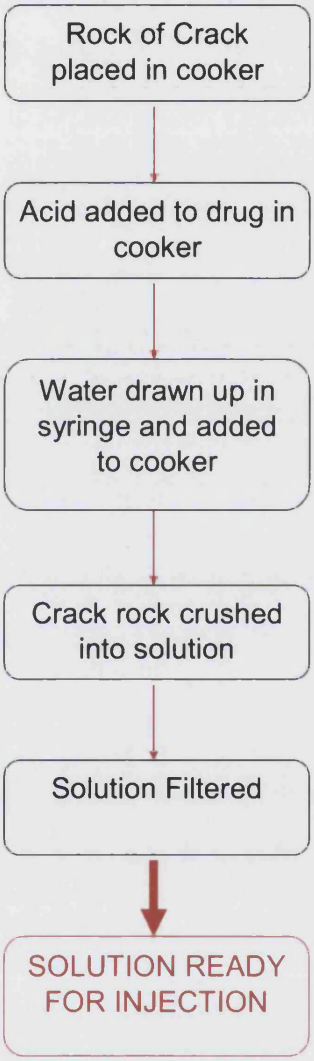


Figure 3. Steps used by six out of eight participants to prepare crack for injection

The other two participants added the acid *after* adding the water to the drug in the 'cooker'.

3.4.4 Speedball injection preparation

All ten of the speedball injections prepared during the demonstrations were prepared using the steps outlined in Figure 4 below:

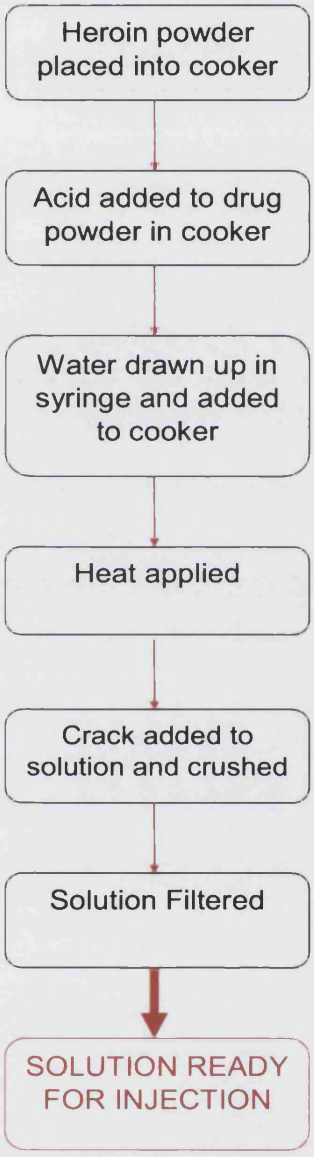


Figure 4. Steps used to prepare a 'speedball' by all participants (n=10)

3.4.5 Equipment used during preparation

The equipment used to complete the same steps of the processes was seen to vary.

Syringe/needle

Irrespective of drug used, the use of 1ml insulin syringe with fitted needle (29G) was prevalent. The Bath and Bristol needle exchanges only dispense insulin syringes in 0.5ml and 1ml, with the 1ml the most popular. DASH also supply 2ml and 5ml syringes upon request, in addition to insulin syringes

Of 64 interviews (one result missing), 55 of the participants used 1ml syringes, three used 0.5ml insulin syringes, and the remaining six (all from Hereford) used 2ml syringes. The variation in syringe use was only seen in heroin injectors, the crack and speedball injectors all chose 1ml syringes (one result for crack injector missing).

3.4.6 Heroin preparation

Cookers

'Cookers' used included tea-spoons, dessert spoons and the bottom of used drinks cans. Forty five participants (96%) chose to use a spoon of either size; the other two used the bottom of a drinks can.

Filters

To filter their injections, 29 (62%) of the participants used material from filters made for hand-rolled cigarettes, 14 (30%) used material from filter tip cigarettes and four (8%) used cotton wool.

Heat source

To heat the injection solutions, a disposable cigarette lighter was used by 41 participants. Three burned pre-injection (isopropyl) alcohol swabs, and one used two matches held together side by side. Two results were missing.

The benefit of using swabs appeared to be the freeing up of one hand which could be used to stir the solution during heating, as was demonstrated by one participant using the needle sheath to stir the solution.

During the preparation only twelve participants stirred the solution, nine of these using the needle sheath; two used the plunger end of the syringe, and the other used a match.

Materials used during preparation

The majority of participants chose to use citric acid (n=37, 79%), eight (17%) chose ascorbic acid as the acid of choice, and two (4%) chose lemon juice.

Water was used from a variety of sources. Thirty (64%) used water from the tap, seven (15%) used water from bottles, includes bottled mineral waters, and tap water stored in a bottle, six (13%) used boiled water and one (2%) stated using sterile water from ampoules (source of these unknown). Data on water are missing for three participants.

3.4.7 Crack cocaine preparation

Seven used spoons as cookers; one used the bottom of a drinks can. To filter, all eight used material from filter-tip cigarettes.

All crack cocaine injectors (n=8) chose to use citric acid for their preparation. Water source varied with three using tap water, one using 'boiled and cooled' water from the kettle, one using bottled water, and one using water obtained from a 'toilet' (it is unknown if this refers to the cistern or the bowl). Data on water is missing for two participants.

The preparation of crack did not involve heating the mixture; therefore no heat source was required.

3.4.8 *Speedball preparation*

As cookers, eight used spoons, the other two used the bottom of drinks cans. To filter, eight speedball injectors used material from filter-tip cigarettes, the remaining two chose material from roll-up cigarette filters.

All participants used citric acid for their preparation. Five used tap water and three used bottled water. Data on water is missing for two participants.

To perform the heroin heating step of the preparation all participants used a cigarette lighter.

3.4.9 *Filter use in detail*

Table 10 Filters used during preparation by the participants at each location

Location	Filter	Number
Bath	Roll-up cigarette filter	2
Hereford	Roll-up cigarette filter	26
	Cigarette filter (portion)	2
Bristol	Cigarette filter (portion)	28
	Cotton Wool	4
	Roll-up cigarette filter	3

Table 10, above, shows the choice of filter used by each participant. The difference in choice of filters is accounted for by the fact that DASH supply their users with Swan roll-up filters for this purpose, neither BADAS nor BDP supplied filters to their users at the time of interview.

The choice of the filters used by the users also appears to influence the way they use them. The use of the filters is not straight forward.

In Hereford, all the users were seen to use part of a filter, rather than the whole thing, this was independent of the filter choice. Roll-up filters had a strip torn from them lengthways to provide a much smaller amount of material for use. With filter tip cigarettes, filter material was pulled out of the end of the cigarette: these measured 7mm wide (the width of a cigarette), by about 2mm thick. The length ripped out varied.

The three users in Bristol who chose to use roll-up filters all used them differently. One used the whole filter; one tore a strip off as done by those in Hereford. The third stripped all the outer 'skin' from the Swan filter and used the

inner core as the filter. The reasoning behind this is unknown, but it is possible it was due to dirt on the outside of the filter.

The majority of the participants in Bristol chose to use cigarette filter material. This was usually removed from the cigarette by cutting the filter with a scissors horizontally, leaving the filter shorter, but otherwise intact (unlike when the material is pulled out, which damages the material left on the cigarette). The cotton wool users removed a piece from one end of a cotton bud, and rolled this into a ball for use.

3.4.10 Heating

The heating of the injections containing heroin (heroin alone, and speedballs) was conducted by all 57 participants. As outlined above, the majority of these participants chose to use a disposable cigarette lighter for this purpose.

For use during the interviews, lighters were purchased from a newsagents shop. These were manufactured by the brand 'Cricket'. Contact with Cricket revealed that these lighters contain a mixture of butane and propane gases, and that under ambient conditions they produce a flame of approximately 850-900°C (Personal Communication, Cricket).

Injections of heroin made using citric acid were heated for 38.7seconds on average (standard deviation = 13.6), and injections using ascorbic acid were heated for 42.3seconds on average (standard deviation = 15.8). Some injectors were asked during the heating how long they would heat for; twenty one stated that it was ready 'when the solution goes clear' or 'when all the solids have dissolved', the others asked (n=3) replied 'when the solution bubbles'.

3.4.11 Materials

The materials that were used by the participants during the interviews were measured and recorded, enabling the comparison of the quantities. During the interviews, problems occurred which rendered some samples unfit for measurement, these included spillages, or other loss of sample from the tubes for later measurement in the laboratory. In total of the 65 people interviewed, 58 interviews had fake drug and acid quantities suitable for measurement.

However, of these 58, eight sets of results were further excluded as the participants had been observed to add additional acid to the preparation during the process after the initial quantity. These results were excluded on the basis that the drug user initially gauged a suitable quantity of acid that was appropriate for the amount of drug they were using, but as the preparation proceeded, they deemed more acid was necessary. The results were removed because the requirement for additional acid may have been falsely suggested by the lower solubility of the drug substitute compared to real drug. This observation is important to the study of drug preparation as it records the titration of acid quantity, depending on the perceived lack of drug dissolution by injectors conducting preparations.

3.4.12 Water source data compiled

Table 11 shows the sources of water used at each location, for all injections prepared (five responses were not recorded).

Table 11. Source of water used to prepare injections by location

Location	Water Source	Number
Hereford	Tap	17
	Boiled and cooled	5
	Bottled (Mineral Water)	5
Bath and Bristol	Tap	21
	Boiled and cooled	2
	Bottled†	5
	Sterile ampoules	1
	Toilet	1
	'Anywhere'	1

†Bottled water includes water from a tap that has been used to refill a bottle, as well as mineral waters. This appeared to be a common practice in Bristol.

3.4.13 Acid with drug detail

There were five different combinations of acid and drug demonstrated. The preparations of crack and 'speedballs' were prepared only using citric acid powder, however, heroin was prepared using ascorbic acid and lemon juice, as

well as citric acid. The interviews of the crack and speedball injectors provided seven and eight sets of results respectively. Twenty-eight users demonstrated the use of citric acid with heroin, this being the most popular acid. The interviews in Hereford included seven with suitable data for analysis of the use of ascorbic acid. The data for the use of lemon juice was only provided from two interviews, therefore is unreliable.

Before the work was conducted, it was hypothesised that there would be some correlation between these factors, for instance, if less water was used more acid would be used, or vice versa.

The amount of fake drug powder, the amount of acid powder and the quantity of water was compared for each combination. To investigate the existence of any correlations both Pearson's correlation and Spearman's rho were used to analyse the results. Pearson's correlation was used to determine the presence of any linear relationships between the sets of data, Spearman's rho was used to assess whether any non-linear relationships between the two data sets exist.

The data set for the heroin injectors using citric acid was the largest and would therefore prove the most reliable; the other data sets only contained eight or less measurements.

Parametric correlations

Table 12 Pearsons correlation coefficient analysis of quantities of materials used by injectors preparing heroin for injection using citric acid (n=28)

		Weight of Heroin	Weight of Citric acid	Amount of Water
Weight of Heroin	Pearson Correlation	1	0.430(*)	0.183
	Sig. (2-tailed)	.	0.022	0.353
Weight of Citric acid	Pearson Correlation	0.430(*)	1	-0.004
	Sig. (2-tailed)	0.022	.	0.984
Amount of Water	Pearson Correlation	0.183	-0.004	1
	Sig. (2-tailed)	0.353	0.984	.

* Correlation is significant at the 0.05 level (2-tailed).

Nonparametric correlations

Table 13 Spearman's rho analysis of quantities of materials used by injectors preparing heroin for injection using citric acid (n=28)

		Weight of Heroin	Weight of Citric acid	Amount of Water
Weight of Heroin	Correlation Coefficient	1.000	0.388(*)	0.341
	Sig. (2-tailed)	.	0.041	0.076
Weight of Citric acid	Correlation Coefficient	0.388(*)	1.000	0.173
	Sig. (2-tailed)	0.041	.	0.379
Amount of Water	Correlation Coefficient	0.341	0.173	1.000
	Sig. (2-tailed)	0.076	0.379	.

* Correlation is significant at the .05 level (2-tailed).

The correlation analysis in Table 12 and Table **13**, above, shows that there is only a very weak positive correlation between the quantities of the three materials used. Therefore there is little to support the hypothesis suggesting a relationship between any of material quantities used.

To further compare the results, the amounts of fake heroin identified as 10 pounds in value were compared alone. This was the closest the results for the fake heroin could be limited to one common factor, yet provide a suitable number of cases for analysis. The ten pound value bag was the most commonly used quantity of heroin- sixteen cases. One case was removed due to the use of a significantly large quantity of citric acid. This resulted in fifteen data sets for analysis.

Figure 5 shows the amounts of citric acid used to prepare ten pound quantities of fake heroin plotted as a scatterplot against the amounts of 'fake' heroin.

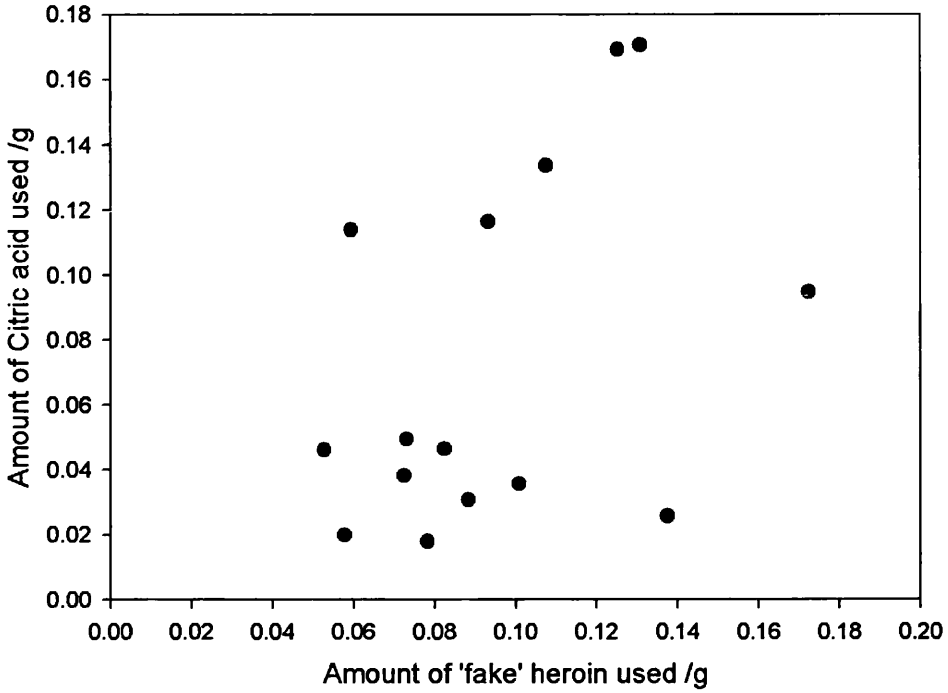


Figure 5 Scatterplot of weight of citric acid used to prepare each £10 in value amount of fake heroin

The correlation is very weak, but it is of a positive nature. Table 14 and Table **15** show statistical analysis of the same data

Parametric correlation analysis

Table 14 Pearsons correlation coefficient analysis of quantities of materials used by injectors preparing a ten pound in value quantity of heroin for injection using citric acid (n=15)

		Weight of Heroin	Weight of Citric acid	Amount of Water
Weight of Heroin	Pearson Correlation	1	0.430	0.071
	Sig. (2-tailed)	.	0.110	0.802
Weight of Citric acid	Pearson Correlation	0.430	1	-0.062
	Sig. (2-tailed)	0.110	.	0.826
Amount of Water	Pearson Correlation	0.071	-0.062	1
	Sig. (2-tailed)	0.802	0.826	.

Nonparametric correlation analysis

Table 15 Spearman's rho analysis of quantities of materials used by injectors preparing a ten pound in value quantity of heroin for injection using citric acid (n=15)

		Weight of Heroin	Weight of Citric acid	Amount of Water
Weight of Heroin	Correlation Coefficient	1.000	0.368	0.225
	Sig. (2-tailed)	.	0.177	0.421
Weight of Citric acid	Correlation Coefficient	0.368	1.000	-0.080
	Sig. (2-tailed)	0.177	.	0.778
Amount of Water	Correlation Coefficient	0.225	-0.080	1.000
	Sig. (2-tailed)	0.421	0.778	.

Again no support is found to suggest any relationship between the quantities of the materials used.

3.5 Discussion of results

3.5.1 Findings from interview situations

The questionnaire, and in particular, the preparation demonstrations provided a large amount of data, from which numerous key points involving the preparation of drug injections were revealed. The data of most importance to this work surrounds that which details how drug injectors learned to prepare injections, and then the preparation details from the demonstration.

3.5.2 Learning to prepare injections

In order to learn to prepare drugs it was important to investigate how current drug injectors prepare *their* injections.

Exploring this subject within the questionnaire part of the interviews revealed that all participants had learned to prepare injections from other

injectors. These injectors included friends, partners or family. No interviewees stated learning from literature, either harm reduction or other sources, nor from any other form of media such as films. The learning process occurs due to the social nature of illicit drug use; to be initiated into using drugs, and to obtain drugs, users will inevitably have to come into contact with other users. These interactions are likely to expose the new user to the habits of other users, and this is likely to include that of injection preparation.

The process of learning to prepare injections from veteran injectors for the laboratory work therefore emulates the process as it occurs within the illicit injecting community. This additionally supports the choice of one-to-one interviews as opposed to focus groups, as this would more clearly imitate reality-observation of the injector, with questioning of details where required.

Demonstration of injection preparation

The demonstration section of the interviews provided a complete characterisation of the injection preparation procedure for heroin, crack and 'speedballs'. The use of saturation technique as the basis for the qualitative assessment of the participant's methods supports this. Once the trends had been noted within the first few interviews, little major variation was seen from these.

Preparation steps

The preparation of the three drugs was observed to be conducted in a distinct series of steps, and they flowed in the same order for the majority of participants. As injectors learn to prepare injections from other users, this would suggest that all injectors learn to prepare injections using the same preparation method as their mentor(s).

Alternatively, it is possible that the order of the steps is used because it is the perceived most effective method, or the quickest method of preparation.

All participants added acid, none tried to prepare without it. This has potential implications for instance, should the heroin on the illicit market change to the soluble salt form, whilst appearing visually similar, injectors could possibly continue to use acids unnecessarily. This would lead to continued, and potentially increased, injecting complications due to acid content.

In order to use the least acid it would be best to put drug into the cooker, then add water and heat the mixture. This would give an indication of solubility of the drug, and possibly indicate the amount of acid required to be added to complete this. In practice this was not seen- one participant did add the water to the drug powder before adding acid, but no heating was performed before the addition of acid. A number of participants (eight of the forty-seven heroin injectors, 17%) were witnessed to add additional acid to the injection mixture during or after the heating stage. This suggests they added a small quantity of acid to the mixture before assessing the solubility upon heating. Once it was clear the drug was not dissolving, they added further acid. This technique will reduce the amount of acid used in total, but the participants still presumed that acid was required in the first instance. Overall, this is the best technique witnessed and a good candidate for harm reduction workers to encourage injectors to use, but it needs to be stressed that the initial quantity of acid added must be kept to a minimum.

The preparation steps occur in the same order the majority of the time, but they are occasionally altered to suit circumstances. For instance, during interviews, one participant stated that if preparing in the open air and it was windy, that he would put the water into the spoon first, so that when the drug was placed into the spoon (next step) it would not blow away. This is unlikely to change the resulting drug injection in any, but with limitations on drug available it was not possible to investigate and confirm this.

Equipment

The choice of equipment showed a degree of variation between participants. This was seen for instance in the choice of cookers where the choice was between dessert spoons and tea spoons, between filters, between needles/syringes and between choice of heat source. It is clear that these are different equipment being used to perform the same purpose, but the variation between them may influence the resulting injection. Different heat sources may result in different temperatures of the solution at the end of heating; a different filter may remove more particles.

Overall, the important factors regarding the equipment is to encourage the use of clean, preferably new and sterile, equipment to prevent blood-borne virus infections, along with other complications such as bacterial and fungal

infections, and vascular damage from blunt needles. Cookers, filters and needles/syringes are all available for supply to IDUs.

Acids and water quantities

Statistical analysis of the quantitative data obtained during the interviews showed very little correlation. There appeared to be no relationship between the amounts of water, acid and drug used during the preparation.

Further interviews could be used to investigate this to a greater degree, but the identification of clear relationships between the three components might not be possible. This would provide support for a theory of individualised injection preparations.

The requirement for acid

All sixty-five participants used an acid during the preparation of their injection. Without addition of acid, it would be impossible to dissolve the drug material in water. No alternative methods were attempted by the participants. This result was the expected outcome and reinforces the anecdotal reports, in addition to the previously published literature.

Fifty-seven described the drug being made to dissolve due to the use of acid; this includes those who stated 'break the drug down', as well as those that were more precise. The other eight participants could be classed as not having an understanding of the reason for its addition.

As previously outlined, all (brown) heroin and crack/freebase is insoluble in water. Therefore these results therefore concur with those of Strang et al.(Strang, Keaney, Butterworth, Noble, & Best 2001). During the interviews, no participants mentioned the use of 'white', or pharmaceutical heroin, which is the hydrochloride salt, as was the case in the Strang et al. London interviews. This suggests a lack of availability of this form of heroin in the interview areas (Bath, Hereford and Bristol), compared to London. This makes it unclear whether participants interviewed for this work know that they do not require an acid to prepare 'white' heroin for injection. It is possible that should this form of the drug become available to them through illicit channels, injectors may continue to prepare it using an acid in a similar manner to the way they currently prepare the insoluble base form.

Acid Preference

During the questionnaire section of the interviews, the questions exploring the choice of acid confirmed information found in some anecdotal reports. The preference of ascorbic acid to all other acids amongst injectors is commonly reported (Derricott, Preston, & Hunt 1999). The majority of the participants chose to use citric acid, although some of them stated that they preferred to use ascorbic acid. They did this as it was the usual preparation procedure having had to resort to citric acid due to the lack of availability of ascorbic acid. The high number of injectors stating they prefer to use Vitamin C powder is of interest, especially considering the difficulty they would have obtaining it.

In contrast to this, some of the participants in Hereford chose to use citric acid over ascorbic acid which was readily available from DASH. During interviews, some participants (data not recorded) stated that it was better to use citric if you had venous access problems. This was on the basis that you would feel burning if you were 'missing' the vein and injecting into the surrounding tissue. This method of detecting extravasation must be considered important to the participants who mentioned it, although it is possible this was only for the fact that they would lose the rapid onset of the drug (the 'rush') effect following intravenous injection, rather than on a safety basis. The injection of solutions into the tissue surrounding veins leads to the formation of 'lumps and bumps'. These remain until the material is removed, with insoluble material likely to remain in the place permanently. The concept that injecting using citric acid that 'burns' (as opposed to ascorbic acid which doesn't burn) to prevent missing veins has never been previously reported. It is possible therefore that the use of citric acid in an effort to prevent extra-vascular injection could possibly reduce adverse events.

It is considered that it is this burning sensation upon injection, whether into, or outside of a vein, that makes injectors consider ascorbic acid the preferable acid. As ascorbic acid is less commonly associated with burning the injectors may, falsely, assume it is safer to use. To date, there exists no *in vitro* or *in vivo* data to suggest which acid is less harmful to injection sites, nor for which acid is safer generally.

A potential benefit of using citric acid is the amount required for use (Scott, Winfield, Kennedy, & Bond 2000). For citric acid to be used to prepare an injection, only a third of the number of acid molecules compared to the number of drug molecules are required. However, for ascorbic acid, an equal number of acid and drug molecules are required. In principle this appears to be of great value, but in practice is likely to be of little benefit.

Firstly, to take advantage of this, the injectors would need to know about molecular weights and understand the concept of 'moles' of materials. This is unlikely for the majority of injectors as it is a complicated concept that many would be unlikely to be familiar. In addition to this, the number of molecules of each constituent is not clearly visible by looking at the amounts of powder (how quantities were observed to be measured during interviews). Different powder particle sizes, combined with the variable amount of diamorphine base present within a drug sample make this impossible to judge by eye. Secondly it assumes that the acids are completely dissociated, which in reality is rarely the case and therefore larger numbers of acid molecules are required to compensate for this.

During the interviews in Bristol, two participants stated they used either lemon juice or citric acid to prepare their injections, and they used both approximately equally. These two participants were in a relationship, and the data from other participants suggests that lemon juice isn't used on a regular basis. Importantly, upon discussion, it was clear they were both aware of the postulated risks of using lemon juice to prepare injections, yet they still continued to do so.

One limitation of the question regarding acid preference is that of the availability of acids. If the participants had only used a very limited range of acids, had had a bad experience from using a poor quality or contaminated batch, or even been given an acid which wasn't what it was stated to be (i.e. given different citric acid but told it was ascorbic acid for instance) they might have suggested they preferred a different acid without fair comparison.

The list of acidifiers *ever* used (section 3.3.10) presents previously anecdotally reported substances. More importantly, it lists many previously unreported substances. No literature article published to date details acids used as acidifiers used by injectors, therefore no figures are available for comparison with these findings. The list is not thought to be exhaustive as there are notable omissions, such as the use of Abdine® (Roberts & Thomson 2000), which is a product only available in Scotland.

It would be expected that the acidifiers with higher reports of use are those which have a better action than those of lower use reports. For instance, vinegar and lemon juice contain suitably high amounts of acids as discussed in the literature review (section 1.10).

Substances reportedly used by just one participant, for instance, bleach, 'Polo' mint sweet and grapefruit demonstrate that injectors are willing to try many substances for the purpose. The fact that they have reported to have been used does not mean they work, and it is possible that their use may have resulted in unique complications, for example, from the injection of bleach.

The results show the level of ingenuity of IDUs. For instance, the use of the coating of the sour sweet (contains citric acid) and of descalers shows that injectors must have considered the nature of the product and deemed, correctly, that it would be suitable for the purpose. Although, very common as demonstrated in the results, the use of lemon juice and vinegar must have required some consideration on the part of a user (or groups of users) to discover their suitability for the purpose.

The most important factor from a harm reduction point of view surrounding these results is that the choice of acidifier appears in no way to be linked to the amount of harm they can possibly cause. Lemon juice had been used by 93% of those interviewed, yet its potential to cause fungal infections has long been suspected. Additionally, 68% of those interviewed had used vinegar on at least one occasion, this presents unknown and unexplored risks, but anecdotal reports suggest it causes headaches upon injection.

Although the results for preferred and regularly used acids suggest a large level of injectors choosing to use what are perceived to be safer substances by harm reduction workers, namely citric acid and ascorbic acid, there does appear to be scope for continued work in the area of educating injectors about the acidifiers they use.

The materials and equipment used by the injectors included those that were available from their needle exchange. This refers to filters syringes, needles and acids. Only in a small number of cases did injectors use alternative equipment, this was demonstrated in the use of filter material from cigarettes. The use of these will have an affect on the final injection, for instance the filter tip cigarette filter may shed more particulate matter into the injection solution than a roll-up cigarette filter. As the majority of exchange users utilised the materials available, this allowed the following laboratory work to be based on the

materials available to them to enable the results to cover the majority of prepared injections.

It is worth noting that the use of the items provided by the exchange may have been used within the interview situation under the assumption by the participants that the interview was actually a 'test' under the auspices of an interview. Therefore, it is possible that the participants may have followed some perceived 'best preparation technique' rather than using equipment or materials they had been warned against using , for instance, lemon juice, or cotton wool. Although this is a possibility, it is not considered to have actually occurred in practice, although it is not possible to confirm this.

3.6 The formulation of a standard injection preparation procedure for laboratory investigations

This section discusses the above results and concludes by describing technique and materials used to prepare injections within the laboratory for further investigation.

3.6.1 Materials

Distilled water (Milli-Q, or water for injections (Fresenius Kabi) for particle counting work)
Illicit drug material obtained from Avon and Somerset Police
Variable:
Acids: Citric acid (Sigma)
Ascorbic acid (Sigma)
Jif® lemon juice (Reckitt Benckiser)

3.6.2 Equipment

1ml disposable insulin syringes (B-D)
Tea spoon (Sunnex brand, Series 311 economy tea spoon)
Disposable Cigarette Lighter (Cricket)

Roll-up cigarette filters (Swan® Extra Slim)

3.6.3 Rationale for materials and equipment used: interpretations from interview data

Drug Material

The quantity chosen for the experiments is detailed at the beginning of the next chapter (Section 4.2). It was based on the quantities of 'fake' drug used during the interviews.

Acid Quantity

Citric acid

To calculate a suitable quantity of citric acid to use to prepare the heroin, the following steps were followed. Firstly, data from the interviews where either fake drug or citric acid powder were spilled were discarded. Secondly, data were also removed where the interviewee had been observed to have added extra acid, during, or after the heating/mixing process. Due to the lower solubility of the fake drug mixture, combined with the fact that addition of extra acid would not improve its solubility, cases where extra acid were added in attempt to dissolve it may have lead to falsely high quantities of acid being added.

To improve the comparison of the data, the results for the use of a ten pound bag of heroin only were considered. With the citric acid users this gave 15 sets of data.

This data indicated that the mean amount of citric acid used was 73.9mg in 0.85ml of water. This would produce a citric acid solution of approximately 87mg/ml.

Table 16. Statistics of injection prepared during the interviews using 'fake' heroin and citric acid (n=15)

	Heroin /g	Water /ml	Citric /g
Mean	.095	.855	.074
Std. Deviation	.034	.248	.054
Minimum	.053	.600	.018
Maximum	.172	1.500	.171

Alternatively, conversion of all acid and water quantities used to a calculated acid solution strength produced the mean solution strength of 92mg/ml.

From these results, the use of **75mg of citric acid** was chosen as the standard quantity of citric acid used to prepare a quantity of heroin equivalent to the average amount of heroin found in a ten pound bag.

Ascorbic Acid

The interview data for the use of ascorbic acid was restricted to a much smaller cohort. Only eight interviewees demonstrated the use of ascorbic acid, and removal of one data set due to spillages reduced this number to seven. Of these seven, only two sets of data were for the use of a ten pound bag of heroin, and these values differed significantly- 151mg, and 19mg. Therefore, the decision was made to compare all the results to obtain values for use in the laboratory.

Comparisons of the means of ascorbic acid and water used suggested a solution strength of 194mg/ml.

Table 17. Statistics of injection prepared during the interviews using 'fake' heroin and ascorbic acid (n=7)

	Heroin /g	Water /ml	Ascorbic /g
Mean	.169	.907	.175
Std. Deviation	.082	.613	.111
Minimum	.070	.450	.019
Maximum	.287	2.200	.335

Calculation of the acid solutions strengths used resulted in a value of 202mg/ml.

Therefore, for laboratory investigations, the value of **170mg of ascorbic acid** was chosen as the standard quantity of ascorbic acid used to prepare a quantity of heroin equivalent to the average amount of heroin found in a ten pound bag.

The quantities of both acids calculated for use in the laboratory experiments were much higher than those used by the only previous work to investigate the use of acids by injectors (Scott, 2000). This used 30mg of citric acid and 60mg of ascorbic acid as the maximum quantities of acid. Given the data from the interviews it suggests that this previous work employed quantities far lower than actually used by injectors, and additionally possibly accounts for the unexpectedly low quantities of drug measured within the injection solutions for that work.

Distilled Water

The majority of demonstration participants (38 of the 65, 58%) stated that they usually used water from the tap to prepare injections. In order to replicate this, water could have been drawn from the tap in the laboratory. However, the use of tap water was not considered to be viable for two reasons. Firstly, the effect of the salts present in the water may affect the prepared injection. This is likely to be the case, and the effect will be specific to the water in each region. Therefore, if the work had been conducted using water from the University taps (supplied by Wessex Water) it could be argued that the results are only relevant to this region. Secondly, the water in the laboratory is occasionally seen to have a faint brown colour due to the piping in the building. This contains unknown contaminants that could have additional adverse effects upon prepared injections.

In order to standardize the injections, the decision to use distilled and deionised water from a Milli-Q purification system was made.

Water Quantity

From the data in Table 16 and Table **17**, the mean quantities of water used by injectors of citric acid and ascorbic acid can be seen to be 0.85ml and 0.90ml respectively. The amount of water used by ascorbic acid injectors was skewed by the use of a high quantity by one participant, and combined with the limited number of data sets; it was decided to use 0.85ml as a standard water quantity for use with both acids.

Syringe

The 1ml disposable insulin syringe was used as it was the most commonly chosen during the demonstrations, and it is the most commonly provided syringe by the three services where the interviews were conducted.

'Cooker' - Tea Spoon

The use of a tea spoon was chosen in preference to that of the dessert spoon. Both were used equally during the interviews and their use appears almost interchangeable. The tea spoon has a smaller surface area resulting in less particle deposition upon the surface during solution withdrawal from the spoon.

The actual tea spoons used for the laboratory injection preparations were the same spoons used by the participants during the interviews. For the laboratory work, the spoon was modified slightly by having a small metal plate soldered onto the end of the handle. This plate minimised the 'rolling' effect of the spoon when being handled, thus reducing the risk of spillage of the injection solutions. This plate would have no effect on the preparation of the injection, nor the injection produced.

Filters

The interviews suggested that approximately equal numbers of users choose to use roll-up cigarette filter material, compared to filter material from a manufactured cigarette. The decision to use Swan® filters throughout the experimental work was taken, as these were the ones being dispensed in a harm reduction capacity by DASH.

In accordance with interview observations, a ripped section of these filters was used in preference to the whole filter as this was demonstrated by all but one participant. Tests in the laboratory demonstrated that the use a whole filter results in a large fluid retention within the filter body of approximately 0.1ml or more. It is possible that injectors have discovered this, and regard this loss of fluid as unacceptable, so they developed the use of a smaller portion. The ripping of filters in this manner is a trend seen amongst roll-up cigarette smokers when they consider these filters to be too big. Figure 6 below shows a filter

ready for use. The filter to be used is end on to the camera, to show the cross-sectional shape, the filter underneath is the remainder of the filter from which it was torn.

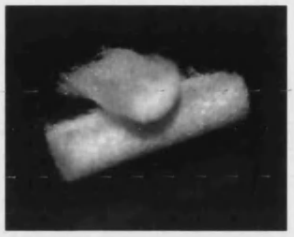


Figure 6. Ripped Swan brand roll up cigarette filter, showing the cross section of 1/3rd of filter (top) as most commonly used by interview participants.

To standardise the drawing of the solution through the filter, the solution was to be drawn perpendicularly to the length of the filter and from the outside edge (as opposed to a ripped inner side).

Additional Equipment

The remaining equipment used was identical to that employed in the interviews, including the lighters.

3.6.4 Use of Lighters

The height of the lighter flame was variable from use to use, depending on the amount of gas present and the time between uses. There was no clear method by which to standardise this, but in the interview situation it was never expressed to be an issue by the participants. The observation of the participants demonstrated that they held the spoon in the tip of the lighter flame with a gentle movement around the spoon bowl. The lighter was often held at an angle, it is unknown if this is beneficial for any particular reason, although excessive use of a disposable lighter can result in melting of the plastic supports for the flint striker wheel, resulting in the wheel springing out and hitting the bottom of the spoon, resulting in spillage of the injection solution.

In line with that observed during the demonstrations, the solutions were heated for at least 30seconds, and until all solids were seen to have dissolved.

3.7 Photographic depiction of heroin injection preparation procedure in the laboratory

The preparation would be conducted following the series of steps as outlined above, as demonstrated by the majority of interview participants. The diagrams on the following pages depict the steps used to prepare an illicit injection for analysis in the laboratory.

Equipment required ready for preparation

Drug, Acid, Water, Syringe, Cooker, Lighter, Filter

STEP ONE:

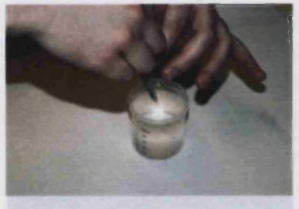
Drug added to 'cooker'

Drug in 'cooker'

STEP TWO:

Acid added to drug in 'cooker'

Drug and Acid in 'cooker'



Drawing up water into syringe

STEP THREE:

Adding water to 'cooker'

Materials in 'cooker'

STEP FOUR:

Heating the mixture

Injection mixture bubbling

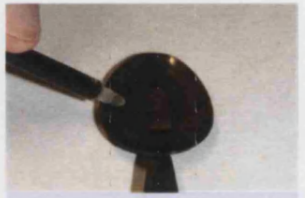
Heated injection mixture

Filter ripped ready for use

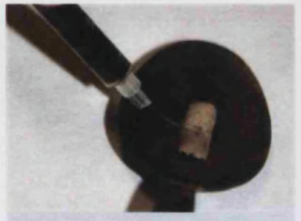
Filter placed ripped side down in the solution

STEP FIVE:

Solution drawn up though filter. Solution drawn up perpendicularly to length of filter



The change of colour of the filter is clear as the final traces of solution are pulled into the syringe.



Note the bending of the needle, this was witnessed on repeated occasions throughout the user demonstrations.

Final injection solution in syringe

4 Laboratory investigations of illicit drug injections

The second section of this project investigated the properties of the prepared illicit drug injections.

The British Pharmacopoeia sets standards to test aspects of formulations to assess factors such as stability, uniformity and ability to deliver accurate doses. It also sets standards which define the safety of a formulation.

Prepared illicit injections would be assessed using methods derived from those used to analyse pharmaceutical injections as set out in the British Pharmacopoeia (2000) where possible. These assays are outlined in the section concerning small volume parenterals, as this is the class that the illicit injections would be placed if they were a pharmaceutical preparation.

Illicit drug injections were not be expected to pass the stringent standards set for pharmaceutical injections, but the results would allow benchmarking against the BP standard and allow comparison as to how far the injections differed from 'ideal' standards.

4.1 Investigations conducted:

Drug sample composition and drug content of the prepared injections
Microbiological content of the prepared injections
Particulate Matter within the injection solution
pH and Osmolality of the prepared injections

Although the interviews investigated the use of crack cocaine in addition to heroin, inability to obtain drug samples for investigation restricted the work to just heroin injections.

The layout of the thesis from this point presents each of these investigations as a separate chapter with an overall conclusions chapter drawing all of the findings together.

4.2 Heroin Samples

In order to produce heroin injections within the laboratory it was necessary to obtain illicit heroin. Samples were obtained from the police forensic science laboratory based in Portishead, Somerset. This heroin originated as seizures from drug users or dealers, at locations within the Avon and Somerset region. The provision of drug samples was organised by the supervisor of this project (JS).

Initially, the drug was obtained as three separate samples, with a total mass of 5.22grams. The samples looked very similar, although they were separate seizures. On the scale used by Kaa (1991), they would be described as a medium brown colour.

4.2.1 Mass and Density Assessment

The fake heroin used within the demonstrations needed to be compared to the real drug powder in order to enable conversion of the mass of 'fake' drug, into that of real drug. The fake 'heroin' employed in the interviews was manufactured from paracetamol tablets as outlined previously. This powder differed from real heroin. It would have a different density and a different consistency, although it is likely that this differs between batches of illicit heroin too.

The amounts of fake heroin used by the interview participants was carefully weighed and recorded from the interviews. These masses are not directly equivalent to that of real heroin powder. To enable comparison of these values to the equivalent of illicit heroin, the volume and the density would have to be measured.

Pharmaceutical science routinely requires the measurement of density of materials. For this purpose, two methods are used- tap density measurement and true density measurement. Both of these methods are approved in the British Pharmacopoeia. Tap density measurements eliminate the gaps between the particles as far as is possible to give a volume measurement of the powder. One disadvantage of the technique is that it requires large volumes of powder, which were unavailable. True density measurement, or pyncometry, uses helium

gas via Archimedes' principle of fluid (gas) displacement and the technique of gas expansion to measure the volume of powder down to gaps in the powder surface to 1 Angstrom ($1x10^{-10}m$). Small powder volumes such as $3cm^3$ can be measured. A major disadvantage with this technique is that it affects the powder being analysed. The use of the gas dries any water from the powder, this will therefore affect any chemicals present in the hydrate form. Additionally, the technique can alter the crystalline form of the chemicals present and alter physical characteristics. This could therefore affect the sample subjected to the test, resulting in inaccurate results in subsequent experiments conducted using this drug material.

To assess the difference between the mass of illicit heroin and the 'fake' heroin used in the interviews, three different methods were used and the results compared. Of importance during this work was the consideration that during all of the interviews all participants measured the amount of drug for preparation by sight. None measured it in any way such as by weighing it or otherwise. This concept formed a basis for the laboratory assessments.

4.2.2 Methods

The first method involved the measuring out of the mean value of 'fake' heroin used in the interviews. This amount of powder was left in a weighing boat, and an approximately equal volume of heroin powder was placed into another weighing boat. This was done through visual comparison of the quantities and relied on the judgement of the person making the measurements. The volume of heroin measured out was then weighed. This was repeated twice more and the results compared to the mass of the 'fake' heroin. Through this comparison, a crude conversion factor could be obtained to translate the mass of 'fake' heroin into a mass of real heroin.

The second method again involved using a sample the mean mass of 'fake' heroin as measured from the interviews. In this case the powder was measured into a 2ml eppendorf tube. The tube was tapped ten times until the powder within formed a level surface. A permanent pen was then used to mark where the powder surface was within the tube. The powder was then removed from the tube, and the tube thoroughly rubbed down inside with clean, dry tissue. Real heroin was then placed into the tube to the level of the line marked.

This powder was also tapped ten times and the level of the powder checked to confirm it still met the line. Once a quantity of heroin equal to the 'fake' powder had been measured to the line, it was weighed. This was compared to the mass of fake and another conversion factor obtained.

The third method was similar to the second, but involved using a known volume of powder rather than mass. To measure the volume, a 10ml conical measure was used. Firstly, the cylinder was weighed and the balance re-zeroed. 'Fake' heroin was then added to the 1ml mark. This powder was tapped ten times to ensure a level surface on the powder at the 1ml mark. The measure was then reweighed and the mass of the 'fake' drug material obtained. The powder was then removed and the cylinder cleaned using a dry tissue. The cylinder was then reweighed again and the balance zeroed. Real heroin was then added to the 1ml line in an identical manner to the 'fake' heroin. A mass of 1ml of real heroin was then obtained. By using the relationship between mass and volume, an approximate of density of each powder could be calculated.

From the results obtained, a crude conversion scale could be drawn up, allowing the conversion of all results of mass of fake heroin into that of real heroin. By dividing this into regions, it also gives an indication of the variation of drug sales in the different areas where the interviews were conducted.

Twenty-two interviews provided weights of 'fake' heroin equivalent to the visual volume of real heroin.
The weighed masses of fake heroin were (in grams): (n=22)
0.0527; 0.0578; 0.0593; 0.0702; 0.0724; 0.0731; 0.0782; 0.0824; 0.0833; 0.0883; 0.0972; 0.1008; 0.1074; 0.1075; 0.1097; 0.1252; 0.1308; 0.1347; 0.1375; 0.1723; 0.1752; 0.2837.

The mean mass is 0.1091g (n=22), however examination of the figures indicates that the largest value (0.2837g) is significantly higher than the rest of the data. Its removal reduces the mean mass to 0.1008g (n=21).

Therefore, 0.1008g or 100.8mg was the weight used for the above experiments. In methods one and two, this mass was weighed out for use in the required manner.

4.2.3 Results

Method 1

This method was performed three times, by two separate people (a, and b).

	a	b
	0.1165g	0.0936
	0.1903g	0.1298
	0.1270	0.0936
Means	0.1446	0.1056
Overall Mean	0.1251g	

The means of the results for each person were calculated then the mean of the mean of these two values was calculated.

This suggested that during visual assessment, the two participants judged that 0.1008g of fake heroin powder resembled 0.1251g of real heroin powder.

Method 2

The mass of real heroin measured into the eppendorf tube to the same volume as that of 0.1008g of fake heroin was 0.1258g.

Method 3

Mass of 1ml of fake heroin = 0.6364g
Mass of 1ml of real heroin = 0.8204g

As 1ml ≡ 1cm^3, the density of each-

Fake heroin 0.6364g/cm^3
Real heroin 0.8204g/cm^3

$\therefore$ 1g of fake heroin = $1.571cm^3$

1g of real heroin = $1.219cm^3$

Assuming constant powder densities,
Mass of fake heroin x 1.289 = mass of real heroin that would have been used

Therefore, this suggests that the equivalent mass of real heroin to that used in the interviews would be 0.1299, or 130mg.

Work by the Independent Drug Monitoring Unit suggests that the average weight of a £10 bag of heroin is approximately 150mg (ranging from 100mg to 250mg). The results therefore fall within the expected range.

130mg was the mass of real heroin used in all experiments.

4.3 Electrospray mass spectrometry analysis of heroin samples and known compounds

4.3.1 Introduction

The illicit heroin samples provided by the Avon and Somerset Police were of unknown content. They were provided with no data on their level of diamorphine content, nor impurity and/or adulterant content. Whether the police laboratories had performed these analyses on the samples was unknown. In an ideal situation this data would have been supplied alongside the samples to eliminate the need for this to be conducted prior to the use of the drug for injection preparation, but samples had been subjected to qualitative confirmation of diamorphine content only for the purpose of prosecution.

The investigation of illicit drug samples by gas chromatography-mass spectrometry is the standard method employed by forensic services, allowing quantification and accurate identification of all components of a mixture. However GC-MS was not available at the University of Bath. HPLC was an alternative method requiring less sophisticated instrumentation with proven validated methods such as the normal phase method developed by O'Neil and

Pitts (1992). Unfortunately, HPLC equipment was not available for use at the time of the sample analysis, although this normal phase method was subsequently tested later during this project during the attempts to develop a method to measure the drug content of injections.

4.3.2 Methodology

To investigate the content of the illicit drug samples, mass spectrometry using electrospray ionisation was employed.

Electrospray ionisation mass spectrometry is a so-called 'soft ionisation' technique and results in very little fragmentation of the compounds in the sample. The 'ionisation' (they are actually charged molecules, not true ions) of the molecules produces peaks only at the molecular weight +1.

The unknown samples were submitted along with known standards to compare the analysis results.

4.3.3 Method

The materials were submitted for positive electrospray mass spectrometry. This required the samples to be dissolved in a water:methanol 1:1 solution, with 1% acetic acid. The acetic acid was added to the sample solution as it enhances protonation and increases sensitivity.

The solution for submission required only 20 to 40 nanograms of the sample substance per microlitre- This equates to 2 to 4 mg per 100ml of solution. The technique is therefore ideal where very limited quantities of samples are available. This was particularly appealing to this project work where very limited quantities of illicit drug samples were available.

The samples were made up to 100ml in volumetric flasks, and a 2ml aliquot submitted for analysis. In total, 17 different samples were submitted, including two from each of the three illicit samples (taken from different regions of the powder) to allow identification of more compounds if the illicit samples themselves were not homogenous.

4.3.4 Instrumentation

The mass spectrometer used was located in the Chemistry department of the University of Bath. The system used was a Waters Autospec M272 system. The equipment was maintained and the samples run by the mass spectrometry technician at the time, Mr. Chris Cryer.

4.3.5 Samples submitted

Samples

Lactose	(Sigma)
Morphine	(Macfarlan Smith)
Codeine	(Macfarlan Smith)
Street heroin (pot A)	
Street heroin (pot B)	
Street heroin (pot C)	
Phenobarbitone	(Sigma)
Diamorphine	(Evans)
Caffeine	(Sigma)
Paracetamol	(Sigma)
Noscapine	(Sigma)
Papaverine	(Sigma)
Acetylcodeine	(synthesised in department)
6-Monoacetylmorphine	(Norfolk and Norwich Hospital)
Street heroin (pot A)	
Street heroin (pot B)	
Street heroin (pot C)	

Materials

Distilled Water	(Milli-Q)
Methanol	(HPLC grade, Sigma)
Acetic Acid	(HPLC grade, Sigma)

4.3.6 *Results*

The inclusion of known standards for analysis alongside the illicit mixtures proved useful in producing confirmation spectra suspected contaminants and adulterants within the illicit samples. The spectra produced by each of the samples are presented in appendix 7.
Figure 7 below is an example spectrum. This was the output for the first sample of illicit heroin, street heroin from pot A.

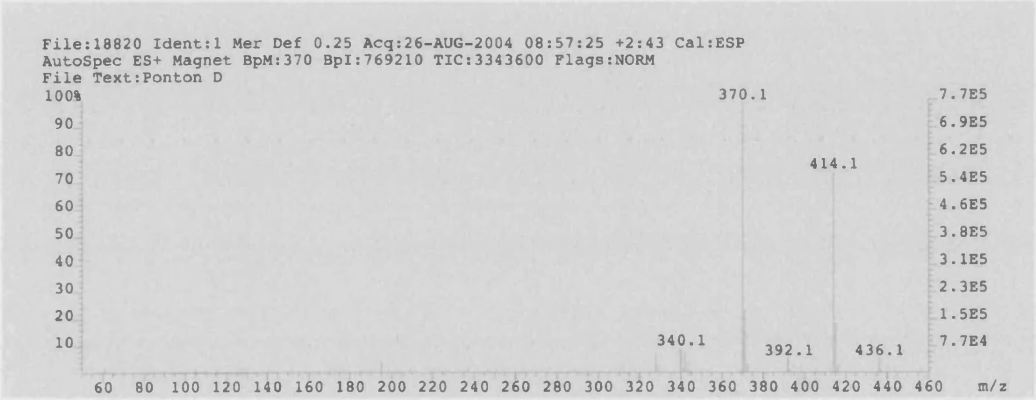


Figure 7. ES-MS spectrum produced by Street Heroin 'A'

This sample spectrum shows five main peaks. The largest peak is that at 370.1, this corresponds to diamorphine. It is the molecular mass of diamorphine (369), plus the mass of a proton. The majority of peaks correspond to the molecular mass of a component present within the sample, plus the mass of a proton. There were smaller peaks that corresponded to the molecular mass of a component, plus the mass of a Na^+ atom. In this example, this is the origin of the peak at 392.1.

Table 18. Identification of the numbered peaks in the spectrum of street heroin 'A'

Peak	Identification
340.1	Papaverine
370.1	Diamorphine
392.1	Diamorphine + Na+
414.1	Noscapine
436.1	noscapine + Na+

The spectrum also includes a number of very much smaller peaks which are more difficult to resolve. These peaks relate to compounds in much lower

relative abundance. These peaks also include isotope peaks, produced by molecules containing an isotope molecule which raises its m/z ratio. The most important here was ^{13}C. The diamorphine peak at 370 was followed by a smaller peak at 371 (23% of original), and much smaller one at 372 (3% of original).

Table 19. Identification of the Smaller peaks present within the spectrum of street heroin 'A'

Peak†	Identification
105	Paracetamol
152	Paracetamol
195	Paracetamol + caffeine
209	Paracetamol
237	paracetamol
328	6-monoacetylmorphine
329	6-monoacetylmorphine (isotope)
341	papaverine (isotope)
342	acetylcodeine
343	acetylcodeine (isotope)
415	noscapine (isotope)
416	noscapine (isotope)
437	noscapine + Na^+

(†approximate m/z values)

Overall, the compounds present within the illicit samples submitted were:

Diamorphine

6-monoacetylmorphine

Acetylcodeine

Noscapine

Papaverine

Caffeine

Paracetamol

Examination of resulting spectra from the illicit samples, alongside submission of standards of other potential components, revealed the samples did not contain morphine, codeine, phenobarbitone or lactose.

4.3.7 Discussion

This analysis produced clear results, and the technique, although not used routinely by forensic analysts, proved to be of great value. The soft-

ionisation of the samples produces molecular weight results for compounds within the mixtures with little or no fragmentation which would result in complex spectra. The other main benefit of this technique is the extremely small amount of sample required- as small as 2mg per 100ml of sample solution. The results allowed firm statement upon the exact compounds present within the samples provided by the police.

Although the results produced by this method were promising, they are not conclusive regarding the suitability of the method to the nature of the work. The submission of both lactose and phenobarbitone standards for analysis, was based on the grounds that they have both been found within illicit heroin mixtures (Kaa 1991;Kaa 1994;O'Neil & Pitts 1992). Both of these compounds, especially phenobarbitone, produced over twenty peaks each. Phenobarbitone also produced a peak at 370.1- the same as diamorphine as seen above. This sample was pure and there was no contamination from any diamorphine, the peak produced must have been produced by a complex of phenobarbitone (molecular weight 232.2). Had phenobarbitone been present within the sample, the abundance peak for species with a m/z ratio of 370.1 would have been raised, suggesting that the diamorphine was more abundant than it actually was. Additionally, and more importantly, it would have made the identification of other compounds within the mixture very difficult to identify- it produced significant peaks at the same m/z ratio as the major peaks for caffeine (195.0) and noscapine (414.1). These problems indicate the advantage of GC-MS, with its ability to separate the compounds prior to mass spectra are run.

Literature searches regarding ES-MS revealed a paper by Selby et al. (Selby 1998). It outlines the possibility of using ES-MS to both identify, and also quantify the content of components within the illicit mixtures. Using drug standards, they produced sample illicit mixtures. Running these in conjunction with known strength standard solutions, they were able to calculate the quantity of compounds within the mixtures.

It was hoped that similar work could have been performed with the aim of quantifying the compounds within the illicit mixtures. These results would then be confirmed using more traditional methods, such as HPLC. If successful, this work would have been the first recorded quantification of illicit heroin samples using ES-MS. Unfortunately, access to the ES-MS system ended in September 2004. This remains an area of potential further work, although it is debatable that the availability of GC-MS would limit its use.

4.4 Determination of drug quantities in illicit drug injections prepared under laboratory conditions using the standardised injection preparation procedure

4.4.1 Introduction

To the injecting drug user, the quantity of drug present within an injection is paramount. Any injection preparation procedure that fails to render the majority of drug bioavailable will be of little value. This is of interest, not only to the user, but also to workers trying to reduce harm during injecting.

In order quantify the ability of each acid to enable the drug material to dissolve, the drug content of the final 'injection' solutions was measured. Investigations of the quantity of drug present in injections prepared using different acidifying agents has only been conducted previously by Scott et al. in 2000. No similar work to this has been conducted according to an extensive search of literature databases. This work built on the previous work through the use of the injection procedure investigated in detail from the injectors and the use methods to explore different properties of the injections. Additionally, new methods were used to repeat previously conducted work, such as the particulate content measurements.

The qualitative identification of drug is a routine procedure for numbers of laboratories. Police forensic services confirm drug content within illicit samples that have been seized in order to enable prosecution. Identification of drugs in illicit samples is routinely carried out by the forensic service using GC-MS. The identification of drugs or metabolites within urine samples are commonly performed by employers and drug treatment services. Often these tests are performed by using simple urine test strips, although in circumstances where a detailed analysis or legally valid results are required, a sample would undergo laboratory tests (Griffin 2003). Identification of drugs or metabolites within urine samples can be conducted using GC-MS, but this is the most sensitive technique. Enzyme immunoassay is more commonly used for initial screening, with GC-MS confirmation conducted if necessary. Urine analysis can

also be carried out using HPLC and capillary electrophoresis (Taylor, Low, & Reid 1996).

Quantitative measurement of a drug within samples is performed less often, and is mainly done specifically by forensic services to compare drug purity trends, and to establish the origin of samples by measuring impurity levels (Griffin 2003). HPLC is used where samples of opiates need to be assessed quantitatively.

4.4.2 Methodology

Prepared injections were subject to assay to measure the content of diamorphine in the solution. This presented a number of obstacles. Firstly, the preparation of the sample for analysis may affect the solubility of the drug, giving a false measurement. Treatment through dilution, or re-dissolving in a different medium may result in an increase or decrease in the quantity of drug measured due to differences in solubility. Secondly, the drug samples are impure and contain many other substances. The presence of unknown compounds complicates the issue of identification and measurement of the drug.

The method used by Scott (Scott, Winfield, Kennedy, & Bond 2000) included the use of capillary zone electrophoresis (CZE) to measure the content of the drug in the injections. Access to a Dionex capillary electrophoresis machine was available. As method development was not an aim of this project the use of an existing method would save time and would reduce validation requirements.

4.4.3 Method 1- Capillary Zone Electrophoresis

Training

The member of staff who had initially owned the Dionex machine had left the department in 1998, and there were no current members of staff who had used the machine. One week was spent at the pharmacy department at Derriford hospital, Plymouth where the same model machine is in regular use. The week taught how to use the machine, how to perform common service procedures and various methods were run including an analysis of morphine within oral preparations.

Following the training, the machine in Bath was prepared for use, and methods used in Plymouth were used to test it. It became apparent at this point that the machine was not working correctly, and there were blockages within some of the tubing lines, leading to pressure build ups and resulting in leaks. The methods were adjusted to prevent these problems from affecting the results albeit through a long winded procedure. This enabled the machine to function correctly; however the results produced indicated that the UV bulb was not functioning at a suitable level. A new bulb was sourced from Dionex, and fitted into the machine. This led to results of a suitable intensity level being obtained from the machine, but unfortunately they were not reproducible. This was unacceptable.

During further assessment and work with the machine to improve the result output, the machine developed a fault with the movement arm (sampling head) that prevented the machine from functioning. At this stage work with the machine was abandoned.

Two methods were employed for use with the CZE during testing. The first was that used for morphine analysis at Plymouth this involved a running buffer of 10mM $Na_2B_4O_7$, 50mM H_3BO_3 and 20mM sodium dodecyl sulphate in water (pH 8.7). Using an unmodified silicia capillary of 75μm internal diameter, 375μm outside diameter and 450mm effective length, 5nl of sample was injected into the capillary by gravity injection. Separation was performed by a constant voltage of 13.5kV, and detection using UV light at 205nm. The run time was 10minutes, with the morphine detected after approximately 7.5 minutes. This method was one the Plymouth staff had previously used to analyse morphine samples, it was provided by Dionex. This method would ensure that reliable results could be obtained from the system before the testing of any actual samples. In order to try and improve the results, the injection was adjusted to raise the sample volume, including use of electrokinetic injection. The separation voltage was raised and UV settings of 254nm and 280nm were also employed.

The second method tested was that of Taylor et al (1996). This used a running buffer of 100mM disodium hydrogenphosphate at pH 6, with a capillary of 50μm internal diameter and 600mm effective length. The capillary was air cooled to prevent the buffer heating up during the separation by the constant voltage of 20kV. A runtime of 20minutes was used. The same UV settings as above were tried.

The outcomes from this work are insufficient to draw any conclusions as to the appropriateness of the methods to the work in question. The previous work by Scott has indicated the later methods suitability. This method was the first choice in the initial instance for this work and would have been used if reliable, repeatable results were forthcoming. Due to the experienced problems, the search for an alternative method was undertaken. The first alternative used HPLC as will now be described.

4.4.4 Method 2 - High Performance Liquid Chromatography (HPLC)

The use of HPLC for quantitative analysis is a first choice for many quantification applications, as it is cheaper than, for instance, GLC, is a relatively easy process and has high throughput. Within the department of Pharmacy and Pharmacology there were functioning machines available for use.

4.4.5 Acetylcodeine and HPLC

Investigation of methods indicate that previous studies in this area were able to achieve adequate separation of the majority of the compounds commonly found in illicit heroin mixtures, but one compound, acetylcodeine, eluted very close to diamorphine, and in some cases co-eluted. The 1986 paper by Kaa (Kaa & Bent 1986) uses the HPLC technique pioneered by Love and Pannell in 1980 (Love & Pannell 1980) and the chromatograph depicted demonstrates how close acetylcodeine elutes to diamorphine. Clear separation of these two peaks was vital in any attempt to quantify the diamorphine through use of HPLC.

Acetylcodeine is formed by the acetylation of codeine that is present within crudely extracted morphine being converted into diamorphine. Acetylcodeine can be present from 1-15% in illicit heroin samples, or 1-80% the relative to the amount of diamorphine (Baker & Gough 1981;Clarke 1986;Kaa 1991;O'Neal, Poklis, & Lichtman 2001). To ensure separation of the diamorphine from the acetylcodeine that was likely to be present within illicit samples, acetylcodeine would be used during the testing of the HPLC method to guarantee that separation could be ensured before real sample analysis was conducted.

There were no standards of acetylcodeine available within the department, and it is not readily available. Standards were only available from Macfarlan Smith Ltd (Edinburgh) the only specialist controlled drug manufacturer in the UK. The cost was prohibitively expensive, so as the Pharmacy department has stocks of codeine, the decision was made to synthesise acetylcodeine from this.

4.4.6 Acetylcodeine synthesis and identification

Method

100mg of codeine base was weighed and placed into a round bottomed flask. To this, 10ml of acetic anhydride and 2ml of pyridine were added. The flask was sealed with a stopper and left for twenty-four hours at room temperature. Acetic anhydride acetylated the codeine, whilst the pyridine maintains a pH basic enough to promote the acetylation reaction, by quenching the H^+ ions produced during the reaction.

At the end of the twenty-four hour period, the flask was placed in beaker of warm water (50-60°C) and a stream of nitrogen gas blown lightly over the surface of the reaction mixture to evaporate as much of the remaining excess acetic anhydride and the pyridine as possible. After two to three hours, 20ml of water was added to the mixture to deactivate any remaining acetic anhydride, by converting it to acetic acid. Sodium carbonate was added to neutralise the acetic acid formed and to raise the pH up to approximately 9.

The solution was then transferred to an evaporating funnel and 20ml of dichloromethane added. The mixture was shaken and the dichloromethane layer (bottom layer) poured off. Another 20ml of dichloromethane was added and the mixture shaken. This layer was added to the previous layer in a beaker. Excess magnesium sulphate was added as a drying agent.

The reaction mixture was filtered through a simple filter funnel with filter paper to remove the magnesium sulphate. The filtered dichloromethane solution evaporated in a rotary evaporator to produce the product, which should have been acetylcodeine, in the base form. There was a possibility that some pyridine may have remained in the product after evaporation. This would be confirmed later.

Identification of product

In order to confirm the identity of the manufactured product, three tests were performed. Firstly, the melting point of the compound was measured. This was found to be 134°C. The quoted reference melting point for acetylcodeine is 134-135°C.

Secondly, a sample of the compound was analysed using the HPLC system under investigation for opiate separation at the time. This method used a Phenomenex C18 Spherisorb 25cm column, 5μm particle size. The mobile phase consisted of 70% Potassium phosphate buffer (15mM; pH 3.5): 30% acetonitrile, running at 1ml/min. A sample of codeine was run injected, then the manufactured product. The product eluted from the column after 5.38minutes, with no other peaks seen. This was significantly later than the codeine which eluted at 1.28minutes. Therefore conversion to another compound had occurred, and no codeine remained. Additionally, a standard of pyridine was run though the HPLC system to record its output peak. This produced a small, poorly defined double peak eluting at 1.64min to 2.49min, demonstrating that the product peak was not produced by pyridine, and that there was no pyridine remaining in the product compound. Diamorphine analysed with the same HPLC system eluted at 5.25minutes.

Thirdly, as the most definitive identification, the compound was analysed by NMR spectroscopy. A small amount of the compound was dissolved in chloroform-*d*3 as the lock solvent, and submitted for analysis.

The NMR equipment used was a Varian Mercury-vx running proton at 399.772 MHz. The samples were referenced to TMS (tetramethyl silane) at 0ppm.

NMR results

Figure 8 and Figure 9 below show the NMR spectrum from the analysis of the sample.

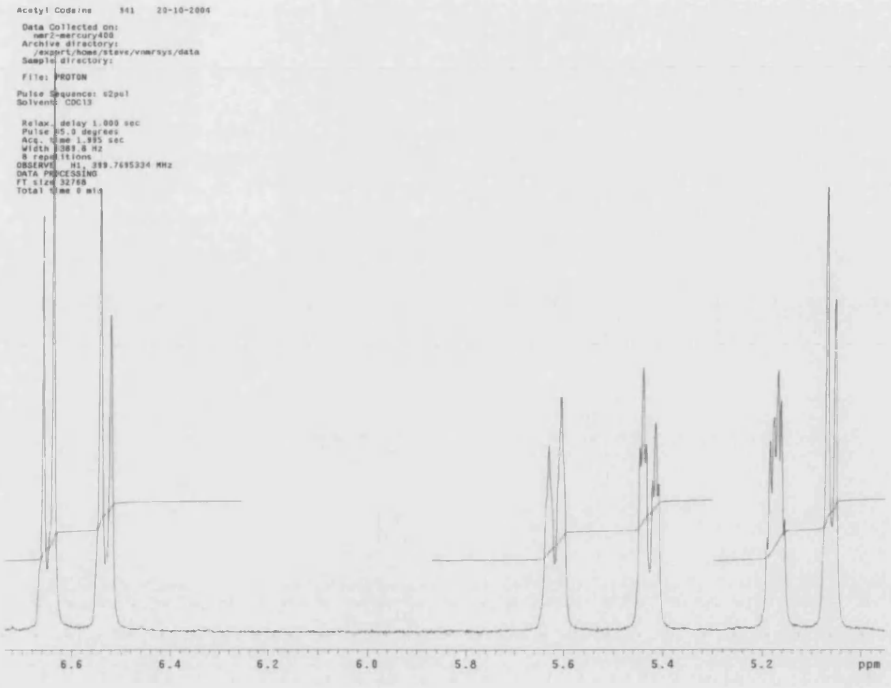


Figure 8 NMR Spectra of synthesised product (1)

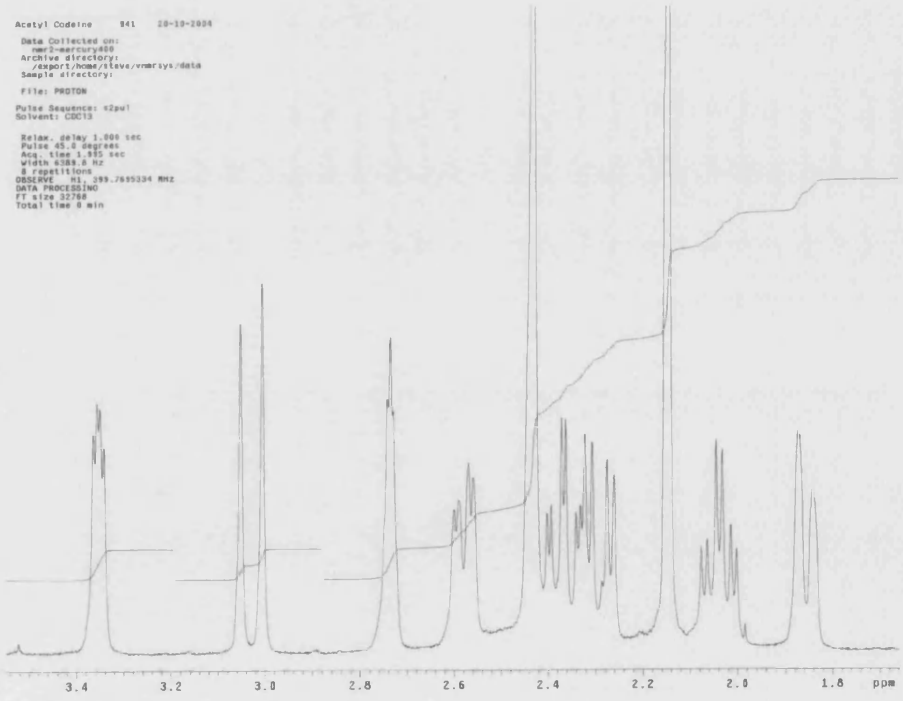


Figure 9 NMR Spectra of synthesised product (2)

The spectrum was compared with a standard spectrum for acetylcodeine and it confirmed the manufactured product was acetylcodeine. The spectrum additionally showed that no pyridine was left within the final product, which had been a possibility.

The preparation method produced the required product, with no contamination from either unchanged starting material, or process materials, specifically pyridine. The process is therefore viable and has been used on subsequent occasions to produce additional acetylcodeine when required. Additionally the manufacture of acetylcodeine, as opposed to purchasing it, saved project funds.

4.4.7 HPLC Methods investigated

HPLC Equipment

Rheodyne manual sample injector with 20µl loop
Jasco PU-1580 Intelligent HPLC pump
Jasco CO-965 Column oven
Jasco UV-1575 Intelligent UV Detector
Varian 4400 Integrator

Detection of the diamorphine was carried out using ultraviolet detection set at a wavelength of 280nm. This is the optimum wavelength according to Clarke and this was confirmed as the lambda maximum by analysis of a diamorphine standard solution using a Cecil 5000 double beam UV spectrophotometer.

Discovery RP-Amide C16 Column Method

The first method attempted was published by the company 'Discovery' (Supelco 2004). One of their 'RP-Amide C16' columns was available for use in the laboratory and these columns have a polar group (amide) which offers less retention of polar compounds and different selectivity to a standard C18 column for which is a recommended replacement. This method would result in less

retention of noscapine and papaverine, leading to shorter run times. If diamorphine and acetylcodeine could be adequately separated, the method would be suitable for use. The column was 150mm with a 4.6mm internal diameter and 5μm particle size. The column was maintained at 35°C. The method used a pH 3.0 phosphate buffer:methanol (80:20) mobile phase, run at 2ml/min.

This assay failed to produce any meaningful results though a problem with the column available.

Kaa/Love and Pannells method

The second method attempted was that used by Kaa in 1986 (Kaa & Bent 1986). The method demonstrated adequate separation of all the compounds within the mixture, allowing quantification of all compounds (heroin, morphine, monoacetylmorphine, papaverine, noscapine, ascorbic acid, caffeine and procaine), except codeine and acetylecodeine, which were quantified using gas chromatography. The HPLC method used a 300mm C18 column, with a mobile phase of acetonitrile and an aqueous solution of 0.75% ammonium acetate. The mobile phase was used in varying compositions from 55:45 to 47:53 depending on the age of the column. The flow rate used was 1ml/min.

To repeat this work, the 300mm column was replaced by a 250mm column as these were available for use without purchase. The column used was C18 with 5μm particle size and 4.6mm internal diameter. The improvements in column efficiency since the publication of the paper suggest this change was justified. The mobile phase was used in a 50:50 composition in the first instance, and then varied to attempt separation.

Gough and Baker method

The third method attempted was that published by Gough and Baker in 1981. This utilised hand-made C18 columns, 250mm in length with 5μm particle size and 4mm internal diameter. The mobile phase consisted of acetonitrile:PIC A reagent (tetrabutyl-ammonium sulphate, 85:15) running at 1ml/min.

Holts 1996 method

The final method attempted was the latest to have been published. This, by Holt in 1996 was the most recent method found and formed part of a study into the size of particles found in illicit heroin seized within the UK rather than solely as a measurement of drug content. The paper states that "levels of acetylcodeine were too low to have any effect on the determination of heroin by HPLC". Given that acetylcodeine is so commonly found in heroin samples, this is considered surprising. It was assumed the authors of the paper had validated this separation.

The method used a 250mmC18 Spherisorb column of 5μm particle size and 4.6mm internal diameter. The mobile phase consisted of potassium phosphate buffer (15mM; pH3.5):acetonitrile, in 70:30 ratio, running at 1ml/min. Detection was performed at 235nm by Holt, so this was attempted alongside the usual 280nm.

Phenomenex Synergi Fusion-RP Method

In a final attempt to find a suitable HPLC method, advice and recommendations on choice of column and attendant method was sought from Phenomenex. This is a company specialise in the development, manufacturing and supply of columns and accessories for the separation, analysis and purification of chemicals and biochemicals. They were willing to select a column that they were confident would produce an adequate separation of the compounds within the illicit heroin, or they would refund the price of the column. They suggested the use of a Synergi Fusion-RP column of 150mm length with a 4.6mm internal diameter and 5μm particle size, with a 30:70 acetonitrile:water mobile phase, running at 1.5ml/min. Detection was again performed using UV absorption at 280nm.

HPLC method outcomes

Attempts to reproduce all of the above methods proved unsuccessful. Peaks could be obtained from samples of both diamorphine and acetylcodeine using all of the methods. Running samples containing both diamorphine and acetylcodeine resulted in one large peak for all methods. Altering running

conditions including changing the mobile phase composition, altering the integrator settings and using a column heater were unable to produce separation of the peaks.

Depicted are below are the results from the method supplied by Phenomenex. The chromatograms are similar to those obtained with all of the above methods, that is, peaks for both compounds alone, a one large peak for a mixture of the compounds. Figure 10, Figure 11 and Figure 12 below show example chromatograms produced using this method.

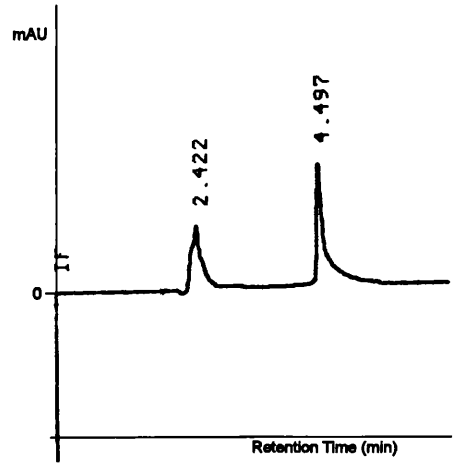


Figure 10. HPLC chromatogram of diamorphine 10µg/ml standard solution.
(Diamorphine retention time 4.497mins)

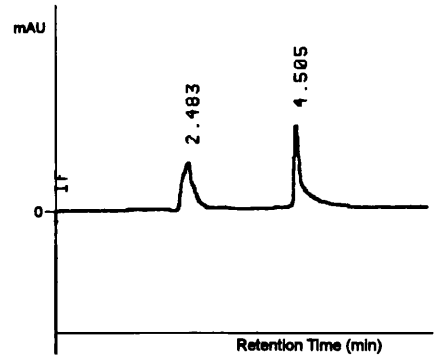


Figure 11. HPLC chromatogram of acetylcodeine 10µg/ml standard solution.
(Acetylcodeine retention time 4.505mins)

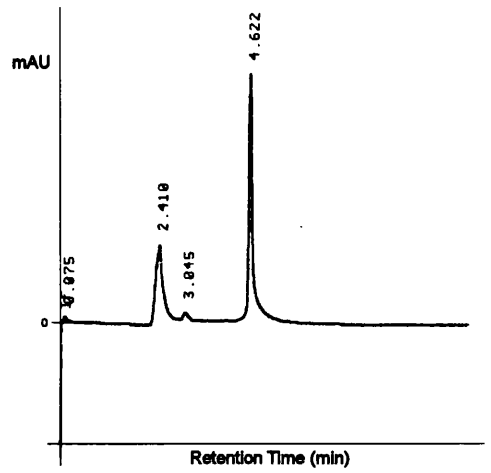


Figure 12. HPLC chromatogram of standard solution mixture 10µg/ml of each drug (Retention time for both compounds 4.622mins)

The diamorphine eluted at 4.497minutes, the acetylcodeine at 4.505minutes. A mixture of the two produced a large peak at 4.622minutes.Clearly the resulting peak was the result of co-elution of the two compounds. Adjustment of the mobile phase ratio down as far as using 100% H_2O, as was able with this specialised column, still showed no ability to separate the peaks.

This method was unsuitable for the separation of the two compounds.

Discussion of HPLC work

The use of HPLC to separate these opiate compounds has been reported in previous work to have been successfully achieved (Holt 1996;Love & Pannell 1980), but none of these methods could be suitably replicated within the laboratory.

The inability to repeat the methods proved to be a complex problem. It is possible that the inability to reproduce the methods of Love and Pannell, as then also used by Kaa could relate to differences in column separation abilities that have changed since their work had been conducted. This however does not explain why the separation abilities reduced, when column technology has improved over time. The separation should have also improved. It is possible therefore that current columns of no longer applicable for this work. The publication by Holt, which used a similar method, stated that 'levels of acetylcodeine were too low to have an effect on the determination of heroin by

HPLC'. They do not state whether they confirmed adequate separation of the acetylcodeine from the diamorphine with this method. This work has proven that separation is not possible, and suggests that Holt was possibly unaware of the presence of acetylcodeine as the two were co-eluting.

For future investigations, newer HPLC methods exist than those attempted, but use technology not available during this project such as diode array detection (Maher, Swift, & Dawson 2001).

4.4.8 Quantitative mass spectrometry

The use of electrospray mass spectrometry in a quantitative matter was considered for the purpose of quantifying the level of diamorphine within prepared injections. It was considered a potentially viable option, but after the retirement of a member of technical staff, the electrospray mass spectrometer was no longer available.

4.4.9 The search for a quantitative method

The requirement for a technique to quantify the drug content of injections had explored the majority of techniques used in the literature for this purpose. As described above, the techniques were unavailable, or could not be replicated.

The use of NMR analysis during the synthesis of acetylcodeine introduced the possibility of the use of it for this work. The availability and access to working equipment in the Department of Pharmacy was of benefit.

4.4.10 Methodology

NMR is principally used for the identification, structural characterisation and purity assay of compounds; however since the spectral data obtained are quantitative, it is possible to use this technique to provide information on the relative amounts of different molecules in a given sample. For well resolved spectra of mixtures, it is feasible to assign which components are present and also how much of each, based on how large the signal is for each of the

component peaks in the spectrum. Comparison of the sizes of these peaks provides a ready means to determine the ratios of the components. Although this method had been used routinely for other purposes, to our knowledge it has not been used to identify and quantify opiates within mixtures before.

4.4.11 Quantitative NMR methods

In order to explore the application of quantitative NMR for opiate mixtures, a series of model heroin mixtures were prepared from known amounts of the seven compounds found in the illicit mixtures by ES-MS analysis.

These mixtures consisted of diamorphine, acetylcodeine, 6-monoacetylemorphine, noscapine, papaverine, caffeine and paracetamol. The mixtures were made using the powder standards measured out to form mixtures with the same percentage of constituents as illicit samples. The powders were carefully homogenised with a small mortar and pestle, then placed into a sample tube and mixed using a vortex whirlimixer for two minutes.

Portions of the powder were taken and placed into an NMR sample tube. Methanol-d_3 was added to dissolve the sample for analysis. The resonance ^{1}H spectra were measured at 400 MHz at first, then at 600 MHz as it was found to give sharper peaks.

4.4.12 Quantitative NMR results

Standards of diamorphine, acetylcodeine, 6-monoacetylmorphine, noscapine, papaverine, caffeine and paracetamol were dissolved in methanol-d_3 and submitted for analysis. The resulting resonance spectra can be found in appendix 8.

Inspection of these spectra revealed that each one had peaks in a region where none of the other components had a resonance, and thus it was thought likely that these resonances could be identified in the spectrum of a mixed sample. This would show both the qualitative presence of that component and also through the use of integration of the resonance, the relative amount of the component with respect of the other compounds.

Samples of laboratory formulated heroin mixtures were then submitted for analysis. These were analysed first at 400Mhz, then 600Mhz. The 600Mhz results produced the spectra, Figure 13. Figure 14 and Figure 15 show expanded sections of Figure 13, with the labelling of the components:

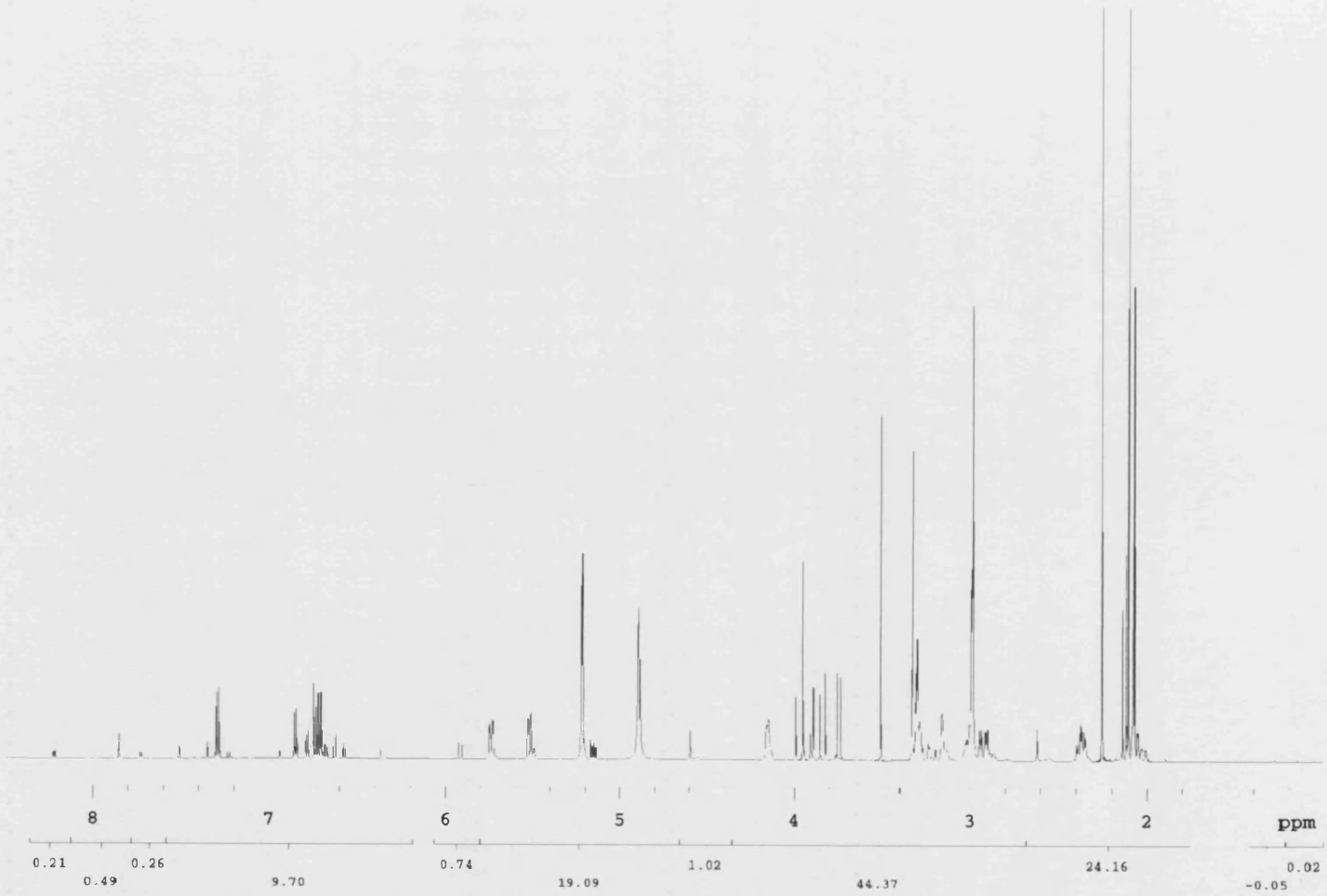


Figure 13. ^{1}H (600MHz, CD_3OD) spectrum of a simulated heroin sample

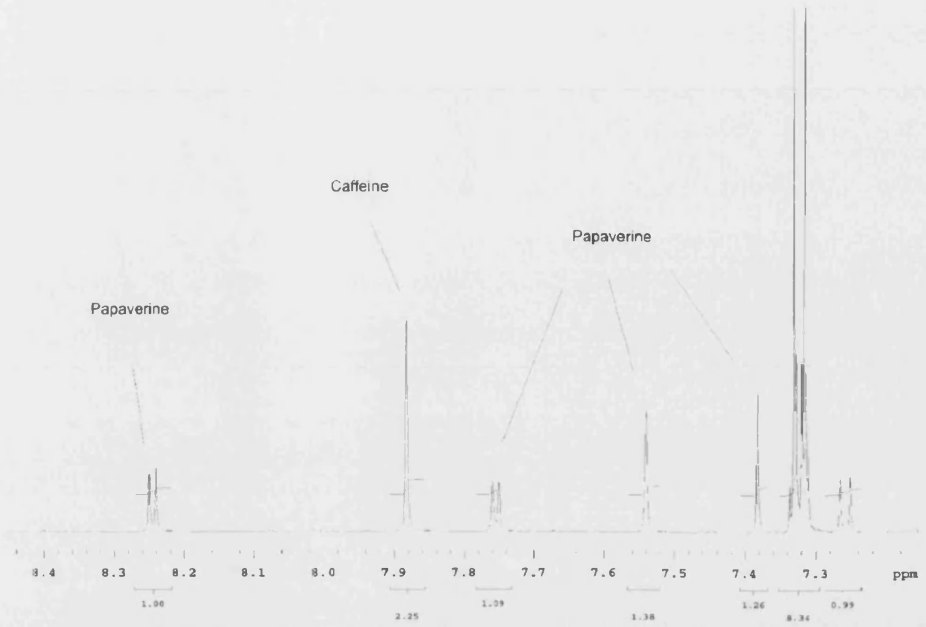


Figure 14. Expanded region of the ^{1}H (600 MHz) spectrum of mix 2 (new)

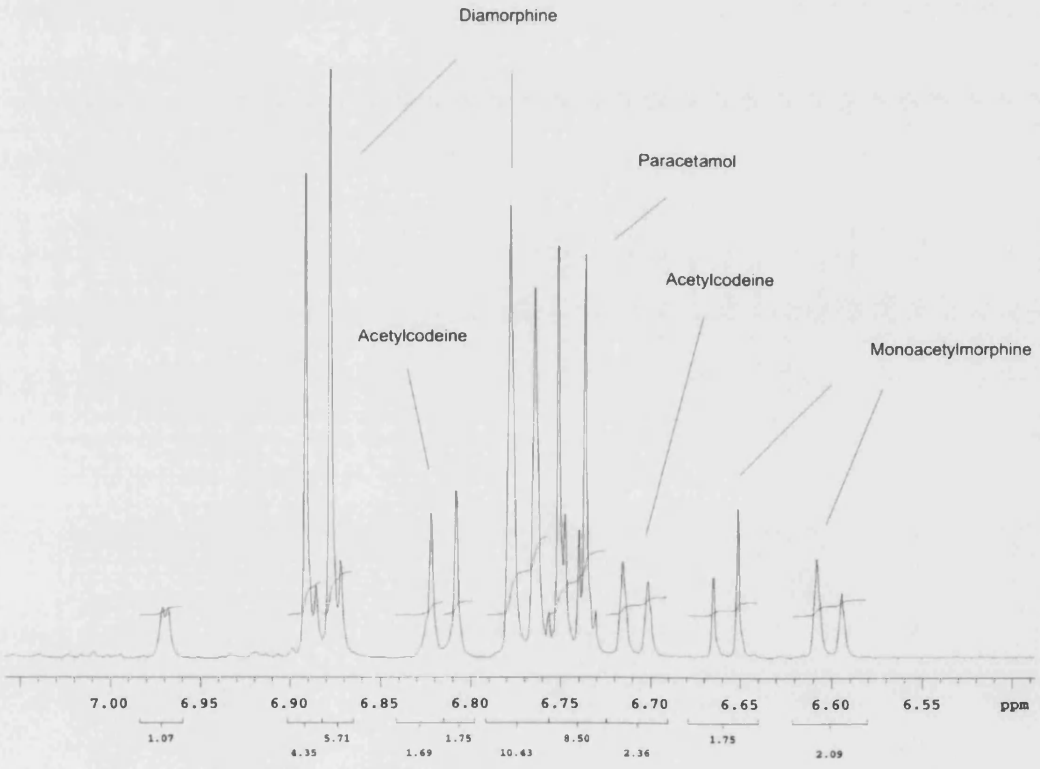


Figure 15. Expanded region of the ^{1}H (600 MHz) spectrum of mix 2 (new)

4.4.13 Analysis of spectra

In order to determine the ability of the method to quantify the compounds present, it was necessary to perform the following calculations:
The number of moles of each of the compounds present had to be calculated from the actual mass of each constituent. This then allowed the calculation of the percentage of each material present, as a percentage of the total number of moles of all compounds present.

The NMR spectra vary depending on the number of protons present on each compound; this was the requirement for the use of moles. By measurement of the integrator trace on each spectrum, you can compare the percentage of compounds present

Sample mix 1

This sample was produced for investigation by the mixing of known quantities of known standards. It was produced on a small scale, the entire sample weighing 10mg. It was accepted that there could be a large degree of error in the use of such small quantities of the constituents, but in order to test the technique in the first instance without wasting large quantities of standards it was necessary. Table 20 below shows the results for the analysis.

Table 20. Expected results versus measured results for constituents of synthetic heroin mixture 1

Constituent	Theoretical % in mixture	Actual mass used, (actual %)	Moles of constituent	% moles	NMR Results Expected	NMR Results Measured
Diamorphine	50%	5.0mg (43%)	0.0135	34.4%	34.4%	36%
Acetylcodeine	10%	1.1mg (9.6%)	0.0032	8.1%	8.1%	0.0%
6-MAM	10%	1.2mg (10.4%)	0.0037	9.4%	9.4%	9.0%
Noscapine	5%	0.7mg (6.0%)	0.0017	4.3%	4.3%	4.5%
Papaverine	5%	1.2mg (10.4%)	0.0035	8.9%	8.9%	18.0%
Paracetamol	10%	1.2mg (10.4%)	0.0079	20.1%	20.1%	23.4%
Caffeine	10%	1.1mg (9.6%)	0.0057	14.5%	14.5%	9.0%

Sample mix 2

This sample was also produced for investigation by the mixing of known quantities of known standards. It was produced on a larger scale, the entire sample weighing 100mg, this would improve the accuracy of the weighing and improve the ability to mix the powders. Table 21 below shows the results for the analysis.

Table 21. Expected results versus measured results for constituents of synthetic heroin mixture 2

Constituent	Theoretical % in mixture	Actual mass used, (actual %)	Moles of constituent	% moles	NMR Results Expected	NMR Results Measured
Diamorphine	50%	(52.0mg, 51%)	0.141	41%	41%	39.4%
Acetylcodeine	10%	(9.7mg, 9.5%)	0.028	8%	8%	14.2%
6-MAM	10%	(10.0mg, 9.5%)	0.031	9%	9%	8.7%
Noscapine	5%	(5.0mg, 4.9%)	0.012	3.5%	3.5%	0.1%
Papaverine	5%	(5.0mg, 4.9%)	0.015	4.4%	4.4%	4.4%
Paracetamol	10%	(10mg, 9.8%)	0.066	19%	19%	19.8%
Caffeine	10%	(10mg, 9.8%)	0.051	15%	15%	12.5%

4.4.14 Discussion

This method was devised in order to allow the measurement of diamorphine within the illicit drug injection solution. The results above demonstrate that the two sample runs conducted were able to measure the quantity of diamorphine present reasonably accurately, with each experiment being within 1.6% of the expected percentage result. This would equate to mix 1

being calculated as containing 5.2mg and mix 2 containing 50.0mg. It is possible that this technique is suitable for the measurements as developed, but further analysis would be required. Further analysis would also need to validate the process to establish whether the differences in the measurements are due to the technique, or due to variation in the samples due to the mixing process.

The measurement of the other constituents was not a primary consideration during the development of the method, but as each component produced distinct peaks, it was made possible. The results for these calculations are not conclusive, however, and it is not possible from this work to suggest whether this technique is suitable for these measurements.

Discussion of all of the methods attempted during the quantitative work along with suggestions regarding future investigations are presented in section 6.1.3.

5 Microbiology of illicit drug injections

The injection of illicit drugs presents a large number of health risks to the users, a proportion of these being due to microbiological infections (Drucker, Alcabes, & Marx 2001). These complications result from the injection of non-sterile solutions. Infections result from viruses, bacteria and fungi present within these injections. Table 22 below shows common illicit drug injecting related infections.

Table 22. Common illicit drug injecting related infections

	Causative agent	
Viruses	Bacteria	Fungi
Hepatitis B	Abscesses	Fungal endocarditis
Hepatitis C	Cellulitis	Fungal endopthalmitis
HIV/AIDS	Septicaemia	Systemic infections
	Bacterial endocarditis	
	Bacterial endophthalmitis	
	Arterial/Venous pseudoaneurysm	

Infections resulting from these three main groups are the most common, but those from other organisms have been reported, including a fatal malaria (protozoan parasite) epidemic in New York (Helpern 1934).

5.1 Candidal infections

Fungal infections from injecting illicit drugs have been recorded since 1971 when Sugar, Mandell, and Shalev first described a case of endophthalmitis. In 1983, Collignon and Sorrell described the first cases of disseminated candidiasis associated with drug use. Since these initial reports, many similar cases have been reported (Dally, Thomas, & Danan 1983;Hoy & Speed 1983;Shankland, Richardson, & Dutton 1986).

The paper of Collignon and Sorrell identified an outbreak that occurred in New South Wales, Australia, in 1982. They described patients suffering from hepatitis followed by eye lesions, skin lesions osteomylitis and costochondritis. *Candida albicans* was cultured from multiple skin lesions in all cases.

Epidemiological investigation found that the seven infected patients were part of a group of nine who had injected identical heroin together one night. Two other injectors present at the same time had remained well. It was found that these two had boiled their heroin solutions before injection. This suggests that boiling of drug mixtures renders the fungi inactive.

5.1.1 Candida endophthalmitis

This is a commonly reported fungal complication of injecting drug use. Endophthalmitis in an illicit drug injector was first described by Sugar et al. in 1971 in the United States, the infection being caused by *Aspergillus sp.*. The most commonly involved organism is *Candida albicans*. Candida endophthalmitis has been recorded in the United Kingdom (Scotland), France and Australia (Gallo, Playfair, Gregory-Roberts, Grunstein, Clifton-Bligh, & Billson 1985;Mellinger et al. 1982;Shankland, Richardson, & Dutton 1986).

In 1985, Gallo et al. published the treatment of ten intravenous heroin injectors with endophthalmitis. Three were confirmed with *Candida albicans* infections, the other seven were treated as presumed fungal infections. Treatment involved regimes of miconazole, flucytosine and amphotericin B. Vitrectomy was performed in the worst cases. The eyesight of only four of the ten improved after treatment. Even in these four, destructive retinal changes had already occurred, leading to poor vision improvement. This case additionally highlights complications resulting from delays in seeking treatment for medical problems, as commonly seen with illicit drug users.

The links between injecting paraphernalia and fungal infections have been investigated by Tuazon, Hill & Sheagren (1974), Mellinger, De Beauchamp, Gallien, Ingold, & Taboada (1982) and Shankland & Richardson (1989; 1986). Tuazon et al. cultured samples of street heroin and injecting paraphernalia looking for organisms implicated in infections commonly seen in injecting drug users. They found that the second most common organism found in street heroin was a fungus - in 30.3% of samples cultured, of which 78.7% were *Aspergillus sp.*. No *Candida* species were found in the heroin, or on the paraphernalia. Mellinger et al. cultured 'brown' heroin samples specifically looking for *Candida albicans*. No samples tested were found to contain the organism, indicating that the drug was not the source of the infections.

5.1.2 *Clostridium novyi*

In the year 2000, an outbreak of a serious infection afflicting injecting drug users occurred in the UK and the Republic of Ireland, with similar cases also affecting one person in both Norway (Maagaard et al. 2000), and Canada (Williamson, Archibald, & Van Vliet 2001). Overall in the UK and Ireland, 109 cases of infection with *Clostridium novyi* were reported, with at least thirty deaths.

Investigations revealed that a batch of heroin was contaminated with the spores of *Clostridium novyi* (Greater Glasgow NHS 2001;McGuigan et al. 2002). It was also found that the drug powder required up to six times the theoretical quantity of acid required to dissolve it, resulting in injection solutions of a very low pH (Greater Glasgow NHS 2001). Epidemiological studies revealed that the injectors who suffered the most severe infections injected subcutaneously or intramuscularly, and that the intravenous users who had suffered infections had missed a vein leading to injection into the surrounding tissue. Intravenous injectors who injected drug from this batch (without missing veins) were unaffected.

The injection of a low pH solution into the non-venous tissue resulted in damage during the prolonged contact; this then provided the ideal growing conditions for anaerobic bacteria such as the *Clostridium*. This outbreak demonstrated that the use of acidifiers by drug users can have serious consequences.

5.1.3 *Research investigating the relationship between injection preparation and infections*

Sources of infection

The occurrence of *Candida* infections has been linked with the use of lemon juice when used as an acidifier. The outbreak in 2000 resulted from bacteria present within the illicit drug material. These are two sources of infections, but other potential sources exist. These include the injecting

equipment, the environment the injection is prepared in and the injector themselves.

Tuazon et al. (1974) investigated the microflora of heroin samples along with injecting paraphernalia. They revealed that *Bacillus sp.* were the most common bacteria found on both equipment and in the drug samples, found on 47% of paraphernalia and in 32% of drug samples. The study also found *Staphylococcus* (32%), *Clostridium perfringens* (31%) and *Escherichia coli* (17%) on the equipment, with *Aspergillus* (26%), *Staphylococcus* (19%), and *Clostridium perfringens* (11%) in the drug samples.

More recently in the UK, McLauchlin et al. (McLauchlin et al. 2002) conducted an investigation of the microflora of heroin samples in response to the 2000 outbreak. *Bacillus* was the most predominant genus of organism found with 95% of samples containing *B. cereus* and 40% containing *B. lichenformis.* In addition to this, 40% of samples were found to contain at least one species of *Staphylococcus.*

Preparation of injections

From the above results, it is clear that bacterial or fungal organisms have potential to be injected when these drugs are used.

The work by Colligan and Sorrell suggested an important point. They indicated that the heating of the injections prepared with lemon juice prevented the injectors contracting fungal infections. The *Clostridium novyi* that caused the 2000 outbreak on the other hand, were clearly able to survive the preparation, including the use of heat and acidifiers.

To date no work has been published that has investigated the ability of organisms to survive the preparation process. The only work conducted in this area is that of Clatts et al. (1999) investigating the ability of HIV-1 to remain viable after being subjected to a typical injection preparation process.

Investigation of the preparation process and the involvement of micro-organisms was therefore prudent during a study of the risks posed by drug injections.

5.2 Investigation into the microflora of prepared heroin injections

5.2.1 Microbiology materials and methods

Training

Prior to the commencement of microbiology work, a University of Bath Department of Biology course on good microbiological practice was attended to ensure good technique, efficiency and safety throughout the following work.

Methodology

Pharmaceutical preparations are subject to the sterility test set out by the British Pharmacopoeia (2000). The object of this method is simply to decide whether a preparation is sterile, or whether it contains viable organisms. The test does not incorporate identification of any organisms that may be cultured. For this project identification of organisms was considered desirable. In addition, the drug solution would possibly have inhibitory actions on the growth of organisms, as heroin has marked antimicrobial properties (Tuazon, Miller, & Shamsuddin 1980).

For these reasons it was decided to use a variation of the method used by McLauchlin et al. This method used techniques to encourage the growth of a wide range of organisms, and allowed their identification. Most importantly, it involves a procedure that removes the heroin from the samples to enable microbiological growth. The second part of the procedure utilises a 'citric acid solubilisation' of the drug in order to remove it from the samples allowing any organisms that would be affected by its presence to grow.

The main issue with conducting microbiological research was identification of colonies produced. Identification of any isolates to the level of McLauchlin's work was ideal, but was not possible due to insufficient laboratory environments and limited expertise. The use of API test strips was considered but was financially limiting with the potential requirement of many different sets of strips for different organisms. Routine Gram staining, along with simple

biochemical tests, such as catalase reactions, were available to allow a level of identification, down to genus level.

In addition to this method, a second investigation was conducted using a method devised during the use of McLauchlin's method for the first stage of work, utilising the same media.

5.2.2 Materials

Agar plates

The agar media used for all of the microbiology method used are detailed within this section.

All seven agar medium plates were made in-house to the following formulae. The agar solutions were made up in a microbiology preparation lab, then sterilised before pouring into sterile disposable Petri dishes. They were kept in cold storage (4°C) until required. No plates older than 14 days were used.

Columbia Blood Agar (CBA):

This is a general purpose medium, containing 5% horse blood, for the isolation of a range of fastidious micro-organisms. This medium combines the virtues of both casein hydrolysate, and meat infusion media resulting in both rapid production of large colonies, as well as clearly defined zones of haemolysis, and good colonial differentiation.

Preparation

Materials

Columbia blood agar base (Oxoid)

Formula:	gm/L	
Special Peptone	23.0	
Starch	1.0	
Sodium chloride	5.0	
Agar	5.0	(resulting pH 7.3 ±0.2)

500ml Distilled Water
5% (25ml) Defibrinated horse blood (Oxoid)

Manufacture

19.5grams of Columbia agar base were weighed out and placed into a 500ml Pyrex® bottle. 500ml of distilled water was added and the mixture boiled to completely dissolve the medium. The mixture was autoclaved at 121°C for 15minutes. The agar solution was allowed to cool to 50°C, and 5% horse blood added aseptically. After shaking, gently to reduce the formation of bubbles, the mixture was poured into sterile Petri dishes.
Appearance of the plates was red due to the blood content.

Neomycin Blood Agar (NeoBA):

This medium is made as CBA, but after autoclaving, 75mg/L of neomycin is added. Neomycin is an aminoglycoside antibiotic active against many strains of Gram negative bacteria, but not Pseudomonas spp. Neomycin is also active against many strains of *Staphylococcus aureus.* NeoBA allows the growth of *Clostridia* and other anaerobes when used in an anaerobic chamber.

Materials

As for CBA agar, along with Neomycin powder (Sigma).

Manufacture

450mg of Neomycin was added to 12ml of sterile water and shaken until dissolved. This solution was then filter sterilised through a sterile disposable 0.2μm filter into a sterile universal vessel.

CBA agar was made as above and after autoclaving and cooling, 10ml of the neomycin solution was added to the agar solution, before the addition of the blood. The solution was mixed gently and poured into sterile Petri dishes.
The plates were red in colour due to the blood content.

Sabouraud agar (Sab):

This is a non-selective acid pH medium for the cultivation and differentiation of pathogenic and non-pathogenic fungi, particularly dermatophytes, and yeasts.

Materials

Formula	gm/L	
Mycological peptone (Amersham)	10.0	
Glucose (Sigma)	40.0	
Agar (Oxoid)	15.0	(resulting pH 5.6±0.2)
500ml Distilled Water		

Manufacture

The ingredients were weighed out in quantities suitable to make 500ml of medium and placed into a Pyrex® bottle. 500ml of water was added to the bottle and the mixture boiled until all components had dissolved. The mixture was autoclaved at 121°C for 15mins and poured into sterile Petri dishes after cooling to 50°C.
The Sab plates were translucent pale yellow in colour similar to nutrient agar.

Fastidious Anaerobe Agar (FAA):

A highly nutritious blood agar base for the isolation of all fastidious anaerobes. This was chosen to replace the BMBH as used by McLauchlin et al.. In discussions, Dr. McLauchlin (Personal Communication) enquiring about the formulation of BMBH, he indicated that he felt that the inclusion of BMBH had had little value. On this advice FAA was chosen to replace BMBH. The formulas are very similar, but using the manufactured powder mix would reduce preparation times.

Materials

Fastidious Anaerobe Agar (Amersham)

Formula:	gm/L	
Peptone mix	23.0	
Sodium chloride	5.0	
Soluble starch	1.0	
Agar No.2	12.0	
Sodium bicarbonate	0.4	
Glucose	1.0	
Sodium pyruvate	1.0	
Cysteine HCl monohydrate	0.5	
Haemin	0.01	
Vitamin K	0.001	
L-Arginine	1.0	
Soluble pyrophosphate	0.25	
Sodium succinate	0.5	(resulting pH 7.2 ±0.2)

500ml Distilled Water

5% (25ml) Defibrinated horse blood (Oxoid)

Manufacture

23grams of the agar mixture was placed into a 500ml Pyrex® bottle and 500ml of distilled water added. The mixture was left to soak for ten minutes before autoclaving at 121°C. After autoclaving the mixture was cooled to approximately 45-50°C and the blood added. After mixing, the mixture was poured into sterile Petri dishes.

The FAA plates were red in colour due to the addition of the blood.

Clostridium Botulinium Isolation agar (CBI):

Clostridium botulinum isolation agar was developed by Dezfulian et al (1981) for the detection of Clostridium botulinum in human faeces. The formula is based on that of McClung-Toabe agar, with the addition of yeast extract to enhance organism growth. McClung and Toabe developed the agar specifically for isolating and detecting Clostridium perfringens in foods.

Materials

Formula	gm/L
Trypticase peptone (Difco)	40.0
Disodium hydrogen phosphate (Fisher)	5.0

Sodium chloride (BDH)	2.0
Magnesium sulphate (Fisons)	0.01
Glucose (Sigma)	2.0
Agar (Oxoid)	20.0
Yeast extract (Oxoid)	5.0

475ml Distilled Water
25ml sterile egg yolk emulsion (Oxoid)

Manufacture

The ingredients for 500ml of solution were weighed out and placed into a 500ml Pyrex® bottle. The mixture was gently heated until all ingredients dissolved, and the mixture autoclaved at 121°C for 15minutes. After cooling to 50°C, the egg yolk emulsion was aseptically added to the solution and mixed thoroughly. The solution was poured into sterile Petri dishes.
The finished agar plates were translucent yellow in colour.

Antibiotic Clostridium Botulinium Isolation agar (ACBI):

ACBI uses CBI agar as the base, with the addition of antibiotics. This medium is used to suppress the growth of other organisms whilst still enabling the growth of *Clostridium botulinum*. This was devised after investigations by Dezfulian et al. (1981), and separately by Swenson et al. (1980), identified resistance to particular antimicrobials by *C. botulinum*. Inclusion of these agents within the media would enable selective growth of *C. botulinum*, particularly by reducing competition from other organisms which are sensitive to the antimicrobials.

Materials

The materials were the same as for CBI agar, with the addition of the following antimicrobials:

Cycloserine (Sigma)	250mg per litre
Sulfamethoxazole (Sigma)	76mg per litre
Trimethoprim (APS)	4mg per litre

Manufacture

Each of the antimicrobials was dissolved in solution in which 5ml contained the required quantity of the agent. 350mg of cycloserine was dissolved in 7ml of distilled water and filter sterilised with a 0.2μm filter into a sterile universal bottle. 106.4mg of sulfamethoxazole was placed in 4ml of distilled water and 10% NaOH added until the drug went into solution. The solution was made up to 7ml with water, and filter sterilised into a sterile universal container. 5.6mg of trimethoprim was placed into 4ml of distilled water and warmed to 55°C in a water bath. 0.05M hydrochloric acid was added dropwise until the drug dissolved. The solution was made up to 7ml, and filter sterilised.

The agar solution mixture was made as above, and after autoclaving and cooling, 5ml of each of the antibiotic solutions was aseptically added to the agar solution, along with the egg yolk emulsion. The solution was then poured into sterile Petri dishes.

The finished plates were translucent yellow in colour.

Polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA):

(*Bacillus cereus* selective agar)

This is a selective medium for the isolation and enumeration of *Bacillus cereus.* Developed by Holbrook and Anderson (1980) for the isolation and enumeration of *B. cereus* in foods, it is able to detect small numbers of the organisms cells and spores in the presence of large numbers of other food contaminants. It allows ready identification of colonies through precipitation of egg yolk and a distinctive turquoise to peacock blue colour surrounding the colonies (Figure 16).

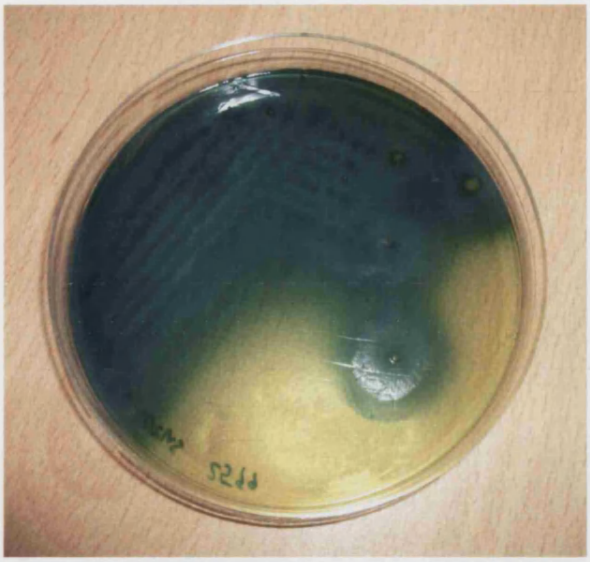


Figure 16. PEMBA plate showing the colour change upon the growth of *B. cereus*

Materials

Formula	gm/L	
Peptone (Oxoid)	1.0	
Mannitol (Sigma)	10.0	
Sodium chloride (BDH)	2.0	
Magnesium sulphate (Fisons)	0.1	
Disodium hydrogen phosphate (Fisher)	2.5	
Potassium dihydrogen phosphate (Fisons)	0.25	
Bromothymol blue (BDH)	0.12	
Sodium pyruvate (Sigma)	10.0	
Agar (Oxoid)	14.0	(resulting pH 7.2±0.2)

475ml Distilled water

25ml sterile egg yolk emulsion (Oxoid)

Bacillus cereus selective supplement SR99 (50000IU Polymyxin B) (Oxoid)

Manufacture

The materials above were measured out in quantities sufficient for 500ml of solution and placed in a 500ml Pyrex® bottle. 475ml of water was added and the mixture gently boiled to dissolve all components. The mixture was

autoclaved at 121°C for 15minutes. The mixture was cooled to 50°C. One vial of the supplement was reconstituted with 2ml of sterile water for injections, and aseptically added to the mixture, along with the 25ml of egg yolk emulsion. The mixture was poured out into sterile Petri dishes.
The PEMBA plates were a translucent lime green colour once poured.

Other Materials

Phosphate Buffered Saline (PBS)

This was made by adding one PBS tablet (Oxoid) to 100ml of distilled water in a bottle, then sterilising by autoclave.

Maximum Recovery Diluent (MRD)

Formula		gm/L	
Peptone	(Oxoid)	1.0	
Sodium chloride	(BDH)	8.5	(resulting pH 7.0±0.2)

This was made up to one litre, then dispensed into 100ml clear glass bottles and sterilised by autoclave.

10% Citric acid solution

Made from citric acid (Sigma) dissolved in distilled water (50g in 500ml), and then filter sterilised.

Clostridium Botulinum Isolation Cooked Meat Broth

This broth was used by McLauchlin et al. (McLauchlin, Mithani, Bolton, Nichols, Bellis, Syed, Thomson, & Ashton 2002) for the cultivation of the organisms in method two of their procedure. They reference a paper stating the origin of this formula; however, the reference actually makes no mention to the formula. Dr. McLauchlin was contacted for the details of the broth formula. The

broth includes meat granules to form an anaerobic environment at the bottom of the vessel as well as providing nutrients to any organisms.

Materials

Formula	gm/L
Trypticase peptone (Difco)	30.0
Yeast extract (Oxoid)	5.0
Dipotassium hydrogen phosphate (Fisons)	5.0
Starch (Sigma)	2.0
Glucose (Sigma)	3.0

500ml Distilled water
Cooked meat medium granules (Oxoid)
250μg of Lysozyme (Oxoid) in 1ml of distilled water, filter sterilised.

Manufacture

The ingredients were measured out in quantities to make 500ml of broth and placed in a Pyrex® bottle. The 500ml of water was added and the solution heated until the all solids were dissolved. One scoop of meat granules was placed in a 25ml thick walled universal container, and 23ml of the broth solution added. The prepared universal vessels were then autoclaved.

On the day of use, each universal was steamed with the lid loose for 15minutes to remove oxygen from solution, and cooled back to room temperature with the lid tightened. One millilitre of sterile lysozyme solution was added to give final concentration of 10μg/ml.

5.3 Investigation method 1

5.3.1 Introduction

The first method used was adapted from the McLauchlin et al. method outlined above.

The method was performed twice, once with 100mg samples of pure heroin in order to replicate the work of McLauchlin using the Avon and Wiltshire Police samples of illicit heroin. This would give a baseline of the organisms within the drug material, which we would be able to compare to McLauchlin's samples (from Merseyside), and then to the results that would be obtained from the heroin injections. This work was repeated in duplicate.

To investigate injections prepared from the heroin samples, 130mg was taken and prepared using sterile citric acid. Due to lack of material, it was not possible to perform the experiment in duplicate. Ideally, this work would have been performed at least in duplicate, then with differing quantities of acid and different acids.

Additional materials

These materials were used in addition to those outlined above. All materials used for the preparation of standard injection, including in this case sterile water for injections (ampoules), and sterile citric acid from Exchange supplies.

1ml syringes (B-D)
10ml syringes (B-D)
30ml syringes (B-D)
60ml syringes (B-D)
Orange (25G) and Green (21G) needles
Syringe filter holders with 0.2μm filter (sterilised by autoclaving)
Glass spreaders (sterilised, or stored in 70% alcohol between use, then flamed)
Sterilised beakers for solutions (citric acid and PBS)
50ml sterile disposable tubes for samples

10% Citric acid solution
PBS
MRD
CBI broth (prepared as described, including steaming and addition of lysozyme)
Plates as previously described

Anaerobic gas jars (2x 15 plate size, 1x 45 plate size) (BBL GasPak)

Gas generating sachets (Oxoid)
Anaerobic indicator strips (BBL GasPak)

30°C incubation environment (To identify fungi and bacteria that grow selectively at this temperature, such a *B. cereus*)
37°C incubation environment (To identify human pathogens)

5.3.2 Part 1

A 100mg sample of heroin was placed into 10ml of MRD and placed on a vortex mixer to form a suspension. 0.1ml of this was inoculated (spread plated) onto two CBA plates, one PEMBA plate and one Sab plate. One of the CBA plates, the Sab and the PEMBA plate were incubated aerobically at 37°C, the other CBA plate was incubated anaerobically at 30°C.

Next, a 1ml sample of the MRD solution was taken and filtered through a sterile 0.2μm filter. This filter was then aseptically removed from the filter holder using a pair of sterilised tweezers, and placed into a universal vessel of CBI broth. This was repeated a second time, but the vessel of broth with the filter was placed in a water bath at 60°C for 30minutes. After the heating of the second broth, both broths were placed in an incubator at 30°C.

After 14 days, both broths were subcultured by spread plating onto ACBI, CBI, NeoBA, FAA and CBA plates that were incubated anaerobically. A second set of CBA plates were inoculated and incubated aerobically. All plates were incubated at 30°C, except the NeoBA plate which was incubated at 37°C. The plates were examined daily for four days, except the Sab plate which was examined for seven days.

5.3.3 Part 2

A 100mg sample of heroin was taken and dissolved in 50ml of sterile citric acid 10% solution. 0.1ml of this solution was spread plated onto each of ACBI, CBI, NeoBA, FAA and CBA plates, which were incubated anaerobically at 30°C, except the NeoBA plate which was incubated at 37°C, and CBA PEMBA and Sab plates which were incubated aerobically at 37°C. All plates were

examined daily for four days for growth, except the Sab plate which was examined for seven days.

10ml of the heroin in citric acid solution was taken and filtered through a sterile 0.2μm filter. This filter was then rinsed with 20ml of sterile citric acid solution, followed by 40ml of PBS. This filter was then aseptically removed from the filter holder using a pair of sterilised tweezers, and placed into a universal vessel of CBI broth. This was repeated a second time, however, the vessel of broth with the filter was placed in a water bath at 60°C for 30minutes. After the heating of the second broth, both broths were placed in an incubator at 30°C.

After 14 days, each of the two broths was subcultured by spread plate onto ACBI, CBI, NeoBA, FAA and CBA plates that were incubated anaerobically, and CBA plates incubated aerobically. All plates were incubated at 30°C, except the NeoBA plate which was incubated at 37°C. The plates were examined daily for four days, except the Sab plate which was examined for seven days.

5.3.4 Investigation of produced injections

After the identification of bacteria present in the drug sample, the presence of these bacteria in a prepared injection was explored. Using 130mg of heroin, injections were prepared and tested using the method above, where they would replace the 100mg of drug powder. As explained above, the following experiment was only performed using citric acid. Ideally, the work would have investigated the use of different acids, varying quantities of these acids and possibly different filters instead of just the Swan® filter used.

Method

The spoon for the preparation of the injection (stored in a beaker of 70% alcohol) was passed through the flame of the Bunsen burner to burn off the residual alcohol. 130mg of drug powder was placed in the spoon. 75mg of sterile citric acid was added to the drug powder. 0.85ml of water for injections was drawn up aseptically from an ampoule using a sterile 1ml insulin syringe and added to the powders in the spoon. The mixture was heated using a cigarette lighter until all solids had dissolved, which took approximately 38 to 40seconds.

A fresh tube of Swan filters was removed from package and the top filter removed and the second one down squeezed up to the opening of the plastic tube. Using a sterile pair of tweezers (stored in 70% alcohol, then flamed before use) 1/3rd of the filter was ripped off and placed into the injection solution. The solution was drawn up into a new sterile 1ml insulin syringe.

For part one of the method above, this injection solution was added into 10ml of MRD. For part two, another injection was prepared as above, and the injection solution added to 50ml of 10% citric acid solution. The methods were then carried out as for the direct samples of heroin powder.

Outline diagrams of method procedures

The following diagrams depict the procedures conducted in the laboratory for the microbiological investigations.

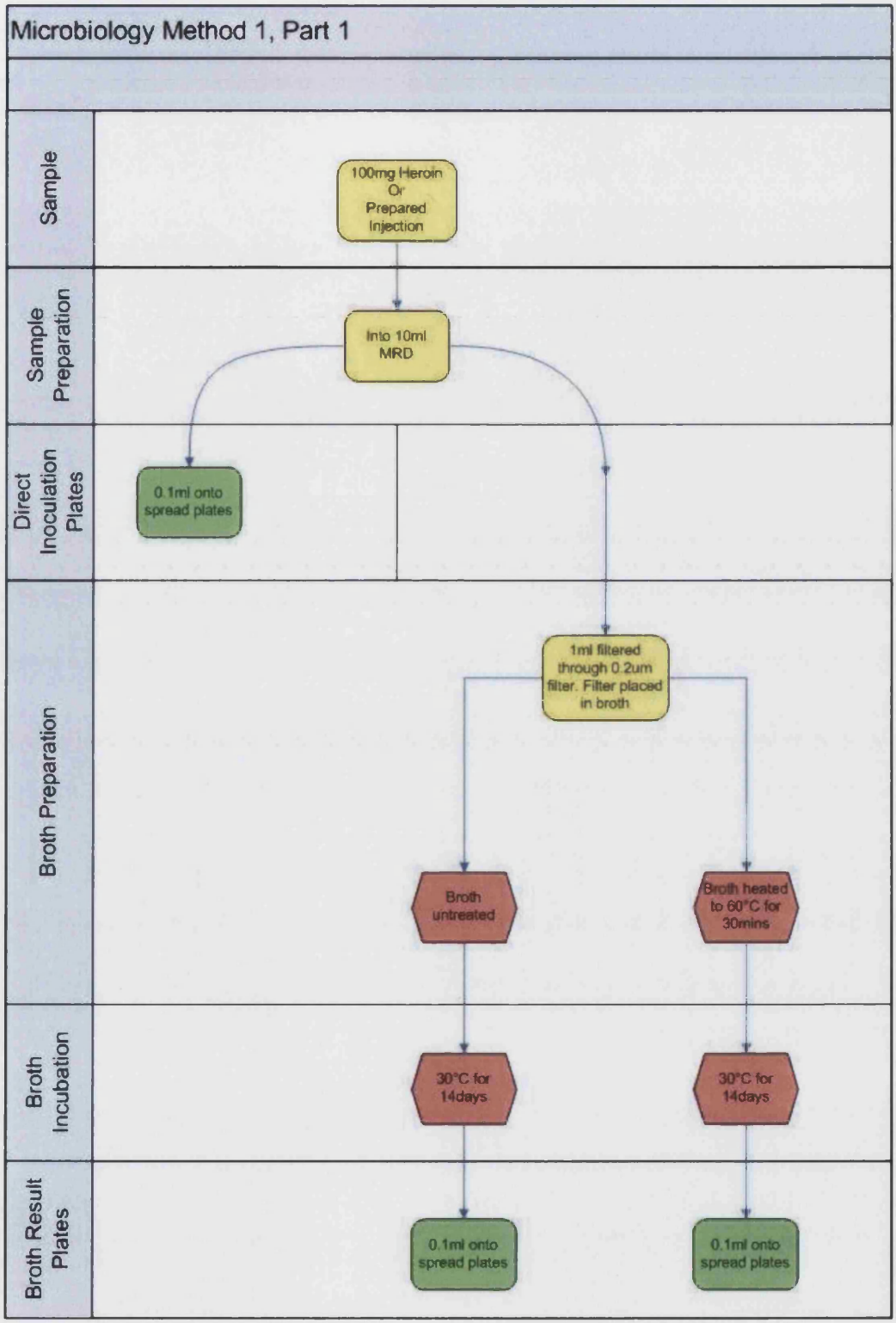


Figure 17 Microbiology method 1, part 1

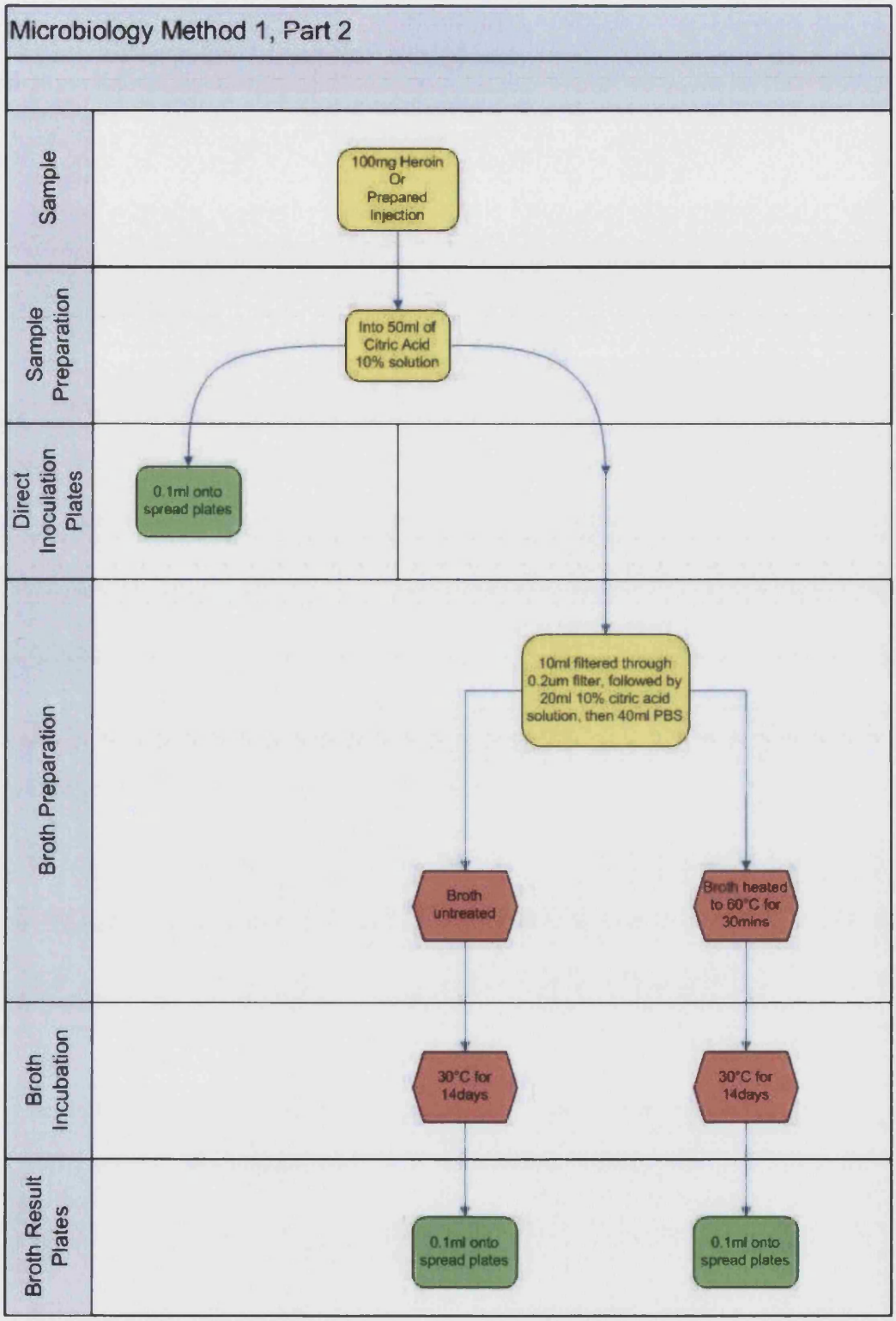


Figure 18 Microbiology method 1, part 2

Validation

To establish the ability of organisms to survive the preparation procedure and remain viable, the methods were repeated using samples spiked with known quantities of certain organisms. McLauchlin et al. used *Clostridium botulinum* (strain R254/02), a strain obtained from an infected wound of an IDU, and *Clostridium novyi,* including a strain obtained from the 2000 outbreak. Both of these organisms are Class 2, which means they 'may cause human disease, laboratory use may present a hazard although laboratory exposure rarely causes infections and effective prophylaxis and therapy are available' (1998). Although they are class 2 organisms, further conditions in the classification system place some class 3 restrictions upon them, specifically regarding the laboratories in which they can be used. Due to these restrictions, combined with increasing concerns about biological terrorism, the use of these organisms within the pharmacy department was not possible. The use of facilities outside the pharmacy department was not possible due to time issues. The work of McLauchlin et al. validated the procedure, and proved that the growth of these organisms under the experimental conditions was possible, but neither of them was recovered from their illicit drug samples. The nature of this work was not to specifically isolate these Clostridium species, but to identify any pathogenic bacteria present within prepared injections. Therefore, validation was conducted using organisms available for use within the laboratory constraints that are known to be the source of infections in injectors. For this purpose, *Staphylococcus aureus* (NCTC 6571) and *Bacillus cereus* (NCTC 2599) were used.

To conduct this for method one, 0.02ml of broth solution (containing approximately 100 colony-forming units, as described in detail in method 2 (Section 5.3.5) was diluted into 0.8ml of sterile water. This was then used as the samples for parts one and two of the method. Specific plates were used for culturing the organisms, namely CBA for the *S. aureus* and PEMBA for *B. cereus.* Growth was confirmed under these conditions.

Further validation was conducted using acid solutions to confirm these did not inhibit the growth of the organisms themselves. 0.02ml of inoculated broth solution above was placed into a spoon with 0.8ml and acid added in powder form. The acids used were citric (75mg) and ascorbic (175mg). They were used from sterile sachets to ensure they did not introduce any micro-

organisms. The solution in the spoon was then heated using a cigarette lighter (as per the standard injection preparation procedure) until the powder dissolved which took approximately 35seconds for each acid. The prepared solutions were then used as the samples and incorporated into the methods as above.

5.3.5 Investigation method 2

This method was devised specifically for this work. It involved the direct inoculation of organisms into all the samples in order to assess their viability after injection preparation. Growth conditions and media were chosen that were most suitable for the organism used. Learning from method 1, filtration of the sample was utilised to remove drug from the samples to prevent any inhibition of growth.

Firstly, a broth of bacteria was cultured from a colony of the standard for the organism. This broth was left static at 37°C for 20 hours. Under these conditions, a broth normally contains between $4x10^8$ to $4x10^9$ organisms per millilitre. To spike the drug cultures, approximately 100 organisms would be added to the injection prior to preparation. The broth would require serial dilution to allow addition of this number of organisms.

Table 23 Serial dilution details

Approximate number of organisms per ml	10^8	10^6	10^5	10^4	10^3	10^2
Broth dilution factor	10^1	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Dilution to be performed on the broth	1/100	1/10	1/10	1/10	1/10	
Actual dilution	0.1ml + 9.9ml	1ml + 9ml	1ml + 9ml	1ml + 9ml	1ml + 9ml	

In 0.1ml of the final (10^2) broth, there should be approximately 10 organisms. Therefore spread plating out of this should result in the growth of approximately 10 colonies. From this, the actual number of organisms per ml can be calculated. This will ensure the spiking of the samples will occur with a known number of organisms. For further spiking experiments, broths will be cultured for 20 hours and measured with a spectrophotometer (Unicam Helios Gamma UV-

Visible Spectrophotometer) to ensure the addition of an equal number of organisms to each drug sample.

The absorbance of the neat broth and that of dilutions of the broth was measured using a spectrophotometer set to measure absorbance at 420nm. Aliquots of the broth were diluted to 75%, 50% and 25% strength. The absorbance of nutrient broth was measured as the zero value. These results were then plotted on a graph of concentration against absorbance. Approximations of the number of organisms in subsequent 20hr broths were possible by measuring the absorbance of the broth, removing the need for serial dilutions and plate counts to calculate the number of Colony Forming Units (CFUs) - the number of viable organisms.

For use, the cultures would then be serially diluted to a solution a suitable strength that would ensure 100 organisms being in approximately 0.02ml of broth solution. This volume was chosen because it was felt too large a volume of broth may adversely affect the production of the injection.

Samples

The inoculation technique was first used to investigate the ability of the organisms to survive heat and acid solutions without drug. 'Injections' were prepared using water for injections alone, 75mg of citric acid in WFI, 175mg ascorbic acid in WFI, and undiluted lemon juice.

Heroin injections were prepared using the standardised injection preparation procedure, using 130mg of heroin. The heroin was prepared using 75mg citric acid as one sample set, and 175mg of ascorbic acid as a second sample set. Sterilising the illicit drug samples to prevent introduction of organisms within them would have been impractical and could have adversely affected the drug material. If a different organism was found in broths or on plates, this would be deemed to have survived the procedure as it was likely to have been introduced by the drug material.

Sample preparation

All work was carried out aseptically on a bench in the presence of a Bunsen burner flame to protect against airborne bacteria. The bench was swabbed prior to work.

The injection was prepared in an identical manner as method 1, observing all aseptic precautions. Before heating, the volume of broth containing approximately 100 organisms was added to the mixture.

The water and lemon juice solutions were heated until they bubbled, heating for at least thirty seconds. The citric and ascorbic acid samples were heated until the acid powder had dissolved, continuing to heat for at least thirty seconds if the acid dissolved quickly. The drug solutions were heated until the drug powder was seen to dissolve into solution which took just over 30seconds.

The solutions were filtered though 1/3rd of a Swan® roll-up filter into a new sterile 1ml syringe with a removable orange needle, not the insulin type as used to draw up the water at the start of the method. This type of syringe would allow the connection of filter holders to produce sample set 2 (below).

Stage 1

This was the direct inoculation of undiluted solutions onto the agar plates. 0.1ml of the prepared injection solution was spread plated onto an appropriate medium for the inoculated organism- PEMBA for *B. cereus,* and CBA for *S. aureus*. This was then incubated for 4 days under conditions to encourage the growth of the organism.

Stage 2

A sterile filter holder containing a 0.2μm filter was used to remove bacteria from the remaining injection solution, and the filter washed with 20ml of 10% citric acid solution to remove any residual heroin from the filter, followed by 40ml phosphate buffered saline to rinse off the acid solution.

The filter was removed aseptically from the holder and inoculated into nutrient broth and incubated at 37°C overnight. The following day, 0.1ml of the broth was spread plated onto the appropriate agar, and this left in suitable conditions to encourage growth of the organism spiked into the drug injection.

Any growth on the plates was identified to establish whether the organism had survived the preparation process.

Controls

Controls were used in stage one for both the organism and the lemon juice solutions. The same volume of broth solution as inoculated into the samples (~0.02ml) was spread plated onto the appropriate agar to ensure the organism was viable. 0.1ml of undiluted lemon juice was also spread plated out to confirm that no organisms were present within it.

In order to confirm the presence of the organism in the broth, 0.02ml of the serial dilution was spread plated onto the appropriate agar for the organism and overnight growth confirmed.

Organisms investigated

Staphylococcus aureus (Commonly found on skin, especially nose and perineum. In context with injection- can cause boils, septicaemia, toxic shock syndrome and endocarditis)

B cereus (most common organism found in heroin samples in McLauchlin's work. Implicated in a wide range of conditions including eye infections, abscess formation, wound infections and septicaemia)

Further investigations

These were conducted during work on the outlined method making use of the surplus drug solution that otherwise would have gone to waste and the filter used to prepare the injections.

Drug in MRD

The preparation of the drug and injection in MRD for part one of the method resulted in approximately 7ml of solution remaining at the end of the experiment. To further explore the presence of viable organisms within this, 0.1ml was placed into 20ml of nutrient broth. This broth was incubated at 37°C for 4 days. This method was not outlined in any previously recorded experimental procedures, but was conducted as the solution would only have been discarded at this point. Given the limited supplies of drug material, this was considered a waste, hence this further exploration of the liquid.

Direct inoculation of 0.1ml of the broth onto CBA plates one incubated aerobically at 37°C and one incubated anaerobically at 30°C, and a Sab plate incubated aerobically at 37°C produced no growth.

Injection filter

The filters used in the spoon to filter the injections may have trapped organisms from within the injection solution. To investigate this, the filters were aseptically removed from the spoon and placed into 20ml of nutrient broth. These were incubated at 37°C for 4 days.

Direct inoculation of 0.1ml of the broth onto CBA plates one incubated aerobically at 37°C and one incubated anaerobically at 30°C, and a Sab plate incubated aerobically at 37°C produced no growth.

5.3.6 Identification of isolates

Any growth found on plates was subcultured by streak plating onto an identical type of agar plate and incubated under the same circumstances as the original plate. By allowing the growth of individual colonies, the colony morphology would usually allow the judgement of whether there was just one, or more than one, types of organism present on the original plate.

Samples from each different colony were examined microscopically using Gram staining. Suspected *Staphylococci sp.* were identified through catalase reaction- the release of oxygen bubbles on contact with hydrogen peroxide solution (3%).

5.4 Results

5.4.1 S. aureus validation

Initial Liquid Samples:
Growth seen on 8 of 8 plates (100%)
All Gram positive cocci, catalase positive.

Broth Samples:

Growth on 6 of 8 plates.

Growth did NOT occur from: Citric acid method 2 steamed Ascorbic acid method 2 unsteamed

Identification

All colonies were identified as *S. aureus* through microscopic examination and catalase tests, except the growth in the citric acid method 1 tube. These were found to be small Gram negative rods, with a weak catalase reaction. Use of API test strips identified these organisms as *E. coli*. Due to incubator maintenance, these broths had been incubated in an alternative incubator alongside the work of another member of staff who was working with *E. coli*, it was therefore probable that this was the cause of this growth. In no other experiments was *E. coli* recovered.

5.4.2 B. cereus validation

Initial Liquid Samples:
Growth on all 8 plates (100%)
All identified as *B. cereus* through blue colour on plates and microscopic examination.

Broth Samples: Growth seen on only 3 plates out of 8
Growth was on citric method 1 unsteamed,
Ascorbic method 1 unsteamed
Ascorbic method 1 steamed
All identified as *B. cereus* through blue colour on plates and microscopic examination.

5.4.3 Ascorbic and citric sachets

Initial Liquid Samples: No growth seen on any plate

Broth Samples: Growth on six out of 56 plates (6/56)

5.4.4 Citric and ascorbic acid from lab stocks

Initial Liquid Samples: One growth out of 32 plates. (1/32, %)

Broth Samples: 17 growths from 56 plates

Identification

Citric method 1 steamed, Aerobic CBA (Plate number 8): motile rods

Citric method 2 unsteamed, Aerobic CBA (15): Gram positive cocci
Citric method 2 unsteamed, CBI (20): Gram positive cocci
Citric method 2 unsteamed, ACBI (21): Gram positive cocci
Citric method 2 unsteamed, Anaerobic CBA (17): Gram positive cocci
Citric method 2 unsteamed, PEMBA (18): Gram positive cocci
Citric method 2 unsteamed, FAA (19): Gram positive cocci

14 Day samples

17 growths on 56 plates

5.4.5 Lemon juice samples

Experiment performed using fresh lemon juice from Jif® lemon shaped container

Initial Liquid Samples: No growth seen on any plate

Broth Samples:

Filtering of the old lemon juice proved troublesome as the filter blocked very easily. Therefore method 2 was impossible to carry out.

Three plates out of twenty-eight produced growth. (3/28, 11%)

Identification:
Fresh Lemon Juice, unsteamed, Aerobic CBA (17): Gram positive rods, anaerobic
Fresh Lemon Juice, unsteamed, FAA (19): Gram positive rods, anaerobic and Gram positive cocci, anaerobic
Fresh Lemon Juice, unsteamed, CBI (20): Gram positive rods, anaerobic

5.4.6 Heroin samples

This work was conducted in duplicate, the sampled being named heroin '1' and '2'.

Initial Liquid Samples: No growth observed. Most likely due to the inhibitory effect of the heroin

Broth Samples: Growth seen on 21 of 56 plates

Identification:
Heroin 1, method 1 unsteamed Anaerobic CBA (3): Gram positive cocci
Heroin 1, method 1 unsteamed FAA (5): Gram variable rods
Heroin 1, method 1 unsteamed CBI (6): Gram positive cocci

Heroin 1, method 2 unsteamed Aerobic CBA (15): FEW Gram positive cocci
Heroin 1, method 2 unsteamed Anaerobic CBA (17): Gram positive rods
Heroin 1, method 2 unsteamed FAA (19): Gram positive rods
Heroin 1, method 2 unsteamed CBI (20): Gram positive rods with spores

Heroin 2 method 1 unsteamed Anaerobic CBA (31): Gram positive cocci
Heroin 2 method 1 unsteamed FAA (33): Gram positive cocci + Gram negative rods
Heroin 2 method 1 unsteamed CBI (34): Gram positive cocci

Heroin 2 method 1 steamed Anaerobic CBA (38): Gram positive cocci

Heroin 2 method 1 steamed FAA (40): Gram positive rods
Heroin 2 method 1 steamed CBI (41): Gram positive cocci

Heroin 2 method 2 unsteamed Aerobic CBA (43): Two types of growth:
Small milky colonies, no haemolysis
Large colonies with haemolysis
Both were Gram positive cocci
Heroin 2 method 2 unsteamed Anaerobic CBA (45): Gram positive cocci
Heroin 2 method 2 unsteamed PEMBA (46): Gram positive cocci
Heroin 2 method 2 unsteamed FAA (47): Gram positive cocci
Heroin 2 method 2 unsteamed CBI (48): Gram positive cocci

Heroin 2 method 2 steamed Anaerobic CBA (52): Gram positive rods
Heroin 2 method 2 steamed (54): FAA Gram positive rods
Heroin 2 method 2 steamed (55): CBI Gram positive cocci

5.4.7 Heroin injections

Initial Liquid Samples:	Growth seen on one plate out of 8. The fact that this was a single colony and was not seen on the replication plate suggests this was external contamination and not significant.
Broth Samples:	No Growth seen

5.5 Microbiological investigation method 2

5.5.1 Results of number of CFUs in overnight broths

Serial Dilutions of the broth solutions incubated at 37°C for 20hours.

B. cereus

Growth from 0.1ml of 10^{-6} culture 3.5 colonies (Average of two plates)
Growth from 0.1ml of 10^{-5} culture 36 colonies

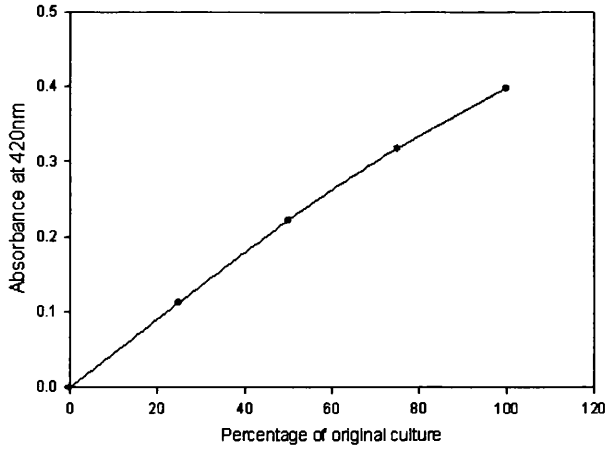
Therefore the concentration of the original broth was approximately $3.6x10^{7}$ per millilitre. Dilution of this of this broth to the 10^{-4} dilution would result in approximately 100 organisms in 27μl.

Spectrophotometer readings (420nm):

Nutrient Broth (0%)	0.0000A
25% *B. cereus* culture	0.1120A
50% *B. cereus* culture	0.2220A
75% *B. cereus* culture	0.3170A
100% *B. cereus* culture	0.3980A

The following graph was plotted:

Absorbance readings of diluted *B. cereus* culture after 20hour incubation



S. aureus

Spectrophotometer readings (420nm):

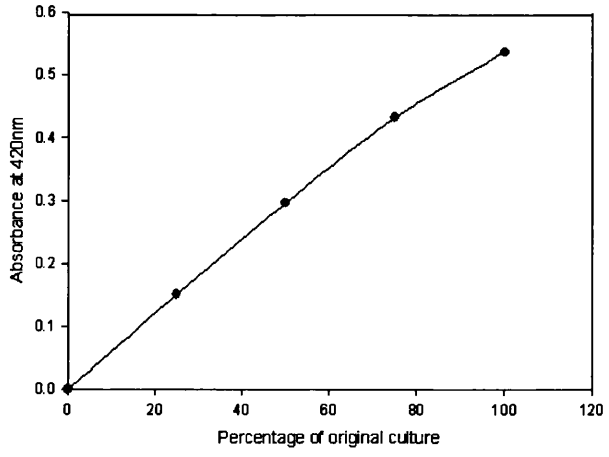
Nutrient Broth (0%)	0.0000A
25% *S. aureus* culture	0.1510A
50% *S. aureus* culture	0.2960A
75% *S. aureus* culture	0.4320A
100% *S. aureus* culture	0.5370A

Growth from 0.1ml of 10^{-6} culture 40 colonies (Average of two plates)

Growth from 0.1ml of 10^{-5} culture approx. 294 colonies

Therefore the concentration of the original broth was approximately $4x10^8$ per millilitre. Dilution of this of this broth to the 10^{-5} dilution would result in approximately 100 organisms in 25µl.

Absorbance readings of diluted *S. aureus* culture after 20hour incubation



5.5.2 Spiked preparation results

Acid Solutions

B. cereus

All samples were cultured on PEMBA medium at 30°C

Results from 0.1ml of neat solution

WFI	No Growth
Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	No Growth
Lemon Juice (undiluted)	No Growth

Controls:

0.025ml of *B. cereus* solution	Growth (approximately 80 colonies)
0.1ml Lemon Juice (unprepared)	No Growth

Broths

After incubation, broth containing filters of the remaining injection solution all appeared clear.

0.1ml of broth spread plate results:

WFI	No Growth
Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	No Growth
Lemon Juice (undiluted)	No Growth

Control: 0.02ml of *B. cereus* in broth Growth

Identification

The growths on the control PEMBA and in the control broth were both confirmed as *B. cereus*.

S. aureus

All samples were cultured on Columbia Blood Agar (5% defibrinated horse blood) at 37°C

Results from 0.1ml of neat solution

WFI	No Growth
Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	No Growth
Lemon Juice (undiluted)	Growth

Controls:

0.025ml of *S. aureus* solution Growth (approximately 150 colonies)

0.1ml Lemon Juice (unprepared) No Growth

Broths

After incubation, the citric acid and lemon juice broths were distinctly cloudy, and the WFI broth had a possible cloudiness.

0.1ml of broth spread plate results:

WFI	No Growth
Citric Acid 75mg	Growth
Ascorbic Acid 175mg	No Growth
Lemon Juice (undiluted)	Growth

Control: 0.02ml of *S. aureus* in broth Growth

Identification

The growth on the control agar and in the control broth were confirmed as *S. aureus.*

The growth from the lemon juice which was taken straight from the bottle indicated that the lemon juice from a bottle opened less than a month earlier was non-sterile. The growth covered over 75% of the plate surface, and was found to be a mixture of Gram variable rods, along with spores. The growth from 0.1ml of prepared lemon juice was restricted to three large colonies. The first of these consisted of Gram variable rods along with spores, similar to that on the unprepared plate. The second colony was found to consist of Gram variable rods. The third colony was markedly different from the others and consisted of Gram positive cocci.

The growth produced by the broth from the citric acid filter covered the entire surface of the plate and consisted of Gram positive cocci, which were identified as *S. aureus* through microscope and catalase reaction test. The broth from the filter used for the prepared lemon juice produced a yellow/gold film over the whole of the agar plate. This film was found to consist of Gram negative rods, with spores.

Heroin injections

The standard injection preparation procedure was used, including the addition of 130mg of illicit heroin powder to the preparation. This was performed with the following acids:
Citric 75mg
Ascorbic 175mg

Results

B. cereus

All samples were cultured on PEMBA medium at 30°C

Results from 0.1ml of neat solution

Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	No Growth

Controls:

0.025ml of *B. cereus* solution	Growth (approximately 110 colonies)

Broths

After incubation, both broths appeared slightly cloudy.

0.1ml of broth spread plate results:

Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	[plate dried up overnight in incubator, result unclear]

Streak plate results

Citric Acid 75mg	Growth
Ascorbic Acid 175mg	Growth

Control: 0.02ml of *B. cereus* in broth Growth

Identification

The growth from the streak plates of the ascorbic acid injection produced a separation of two apparently distinct organisms. There were colonies with a fine 'cotton wool' appearance, which produced a slight blue tinge to the PEMBA agar, not as obvious as a culture of *B. cereus*, but suggesting they were related to *B. cereus*. The second colonies observed were very small white colonies. These produced no blue colour from the agar.

Microscopic examination of these colonies found that they were different organisms. The cotton wool type colonies were observed to be Gram variable rods. The white colonies were Gram positive rods, with spores. Both organisms were found to be catalase positive.

The injection produced using citric acid a homogenous culture of white colonies. Under the microscope, they were found to be Gram positive rods with spores. These were also catalase positive.

S. aureus

All samples were cultured on CBA medium at 37°C

Results from 0.1ml of neat solution

Citric Acid 75mg	No Growth
Ascorbic Acid 175mg	Growth (Two colonies)

Controls:

0.025ml of *S. aureus* solution Growth (approximately 100 colonies)

Broths

After incubation, both broths appeared slightly cloudy.

0.1ml of broth spread plate results:

Citric Acid 75mg	Growth
Ascorbic Acid 175mg	Growth

Streak plate results

Citric Acid 75mg	Growth

Ascorbic Acid 175mg Growth

Control: 0.02ml of *S. aureus* in broth Growth

Identification

The growth from the spread plate of broth from the ascorbic acid injection grew two separate colonies, as shown in Figure 19.

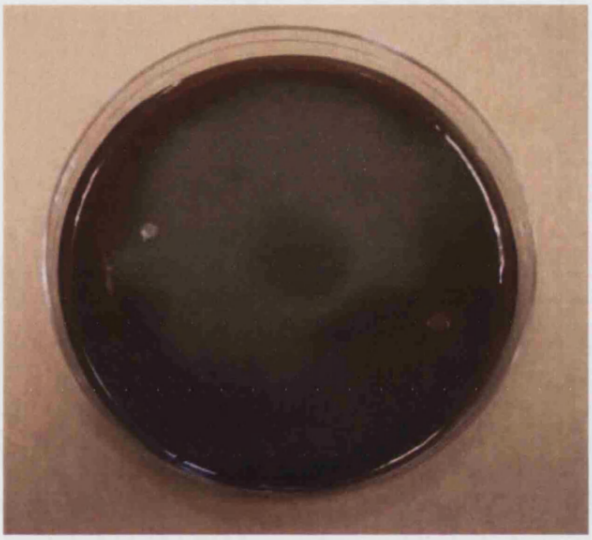


Figure 19 Colonies grown on the CBA spread plate of ascorbic acid solution spiked with *S. aureus*

They were both identified as Gram variable rods, even though the colonies appeared different. This is possibly due to the effect of the injection solution upon the colony growth. No growth of *S. aureus* was evident.
Additionally, the effect of the injection solution upon the blood within the agar can clearly be seen in the photograph. The greying of the agar was clearly caused by the solution, and is especially evident where the initial sample was dropped onto the agar as the round circle in the centre.

The spread plates of the broth solutions both produced golden growths covering the entire surface of the plate. The growths on both plates appeared visually to resemble the growth of *S. aureus*. To investigate this further, the broths were streak plated out to assess whether the cultures within the broths were a mixture of organisms or one single type.

The streak plates from both plates indicated that both broths contained homogenous cultures. The cultures produced from the ascorbic acid injection consisted of catalase positive Gram variable rods. The cultures from the citric acid injection also consisted of catalyse positive Gram variable rods, possibly with a larger number of Gram positives.

5.6 Discussion

The literature review has demonstrated that bacterial and fungal infections contracted through the use of illicit injections are of common occurrence. Not only do they lead to severe morbidity and mortality, but they increase they increase the workload of medical services that have to provide treatment. Viral infections including HIV/AIDS and hepatitis C result in long term health complications, which have high treatment costs. For this reason, a large amount of harm reduction information is directed at users to reduce their spread. Bacterial and fungal infections are less publicised, possibly on account of the acute, rather than chronic, nature of their infections. However, they are both responsible for afflictions and deaths.

5.6.1 Method 1

This section of the microbiological investigation repeated the work of McLauchlin et al. using the same methods and equipment. The one difference from the original work was the culturing of the broth after fourteen days only, rather than at seven and fourteen days. This was conducted due to initial tests suggesting that contamination of the samples could occur during the sampling at seven days despite strict precautions being undertaken to prevent this occurring. It could have been possible to prepare separate broth samples for culturing at seven days, but this would have added to the complexity of the work by doubling the number of broths prepared. Although this was a deviation from the original procedure, it was felt that the most dominant organism would be the only one present at 14days, and this was the one desired for identification. The risk of introducing an extraneous organism from the environment was too great.

The validation of the method using the organisms prepared within acid solutions to confirmed the ability of the organisms to grow in the presence of the acids which would be used to prepare drug injections. This validation confirmed that both *B. cereus* and *S. aureus* were able to grow from acid solutions prepared in a similar manner to illicit injections, minus the drug, from the initial broth samples.

The isolation of these organisms from the incubated broths was less successful. *S. aureus* remained viable within the broth from six out of eight of the acid solutions. The growth of *B. cereus* from the incubated broth samples was seen in only three out of the eight samples. Although unsuccessful, it is not particularly significant. The organisms were confirmed present within the solutions immediately after preparation. This was the most important result. It demonstrates the solutions still contained viable organisms at the point at which it would be injected. Methodologically, it is possible that the conditions were not ideal for the growth of these organisms, this is understandable as the broth formula and its treatment was designed for the cultivation of anaerobic organisms, specifically *Clostridia*.

Sterile sachet results

The sterile sachets made for supply to drug users, are 'sterilised by gamma radiation at a dose that guarantees sterility' (Personal communication, Andrew Preston, 2004). Therefore, the investigation of these sachets should have produced no results. The spread plates from the initial liquid samples confirmed this and no growth was seen. The results from the broth samples were unexpected. Growth occurred on three plates on each of the citric and ascorbic acids (method 2, unsteamed).

This should not have occurred. If the sterility of these sachets is to be assumed, then the growth indicates contamination introduced during the sample preparation. The manufacturers of the sachets maintain they are sterile, therefore the results were assumed to be false and introduced during the laboratory analysis. Following these results, the conduction of the laboratory work was reassessed to attempt to identify the source of the error and eliminate it from future work.

Acids from lab stock

These were sampled from the pots used for experimental work within the laboratory. The aim was to attempt a pseudo-analysis of citric and ascorbic acid samples that had been exposed to the air and objects (spatulas). These acids are available for purchase in large quantities, for instance, Care branded citric acid which comes as a 50g quantity in a plastic bag within a cardboard box when purchased from pharmacies. This is sold to injectors requesting it. This container contains enough acid to prepare many injections. The powder itself is not sterile when purchased, it is classed as for 'culinary use', and once it is opened for use it will be liable to contamination from airborne micro-organisms. In addition, organisms may be introduced by implements the users use to remove acid for preparation, or fingers used to pick 'pinches' of acid. In lieu of collecting samples of acids in use by users, the acids used for laboratory work were investigated for their microbiological content. These acids were a poor substitute on the basis that they had been treated very carefully during use, only coming into contact with clean laboratory spatulas, and stored in a cool, dark, dry environment. Therefore, the only means of potential contamination was from airborne organisms, so this work only provided an insight into organisms that could potentially survive in the acid material before its use to prepare injections. Investigation into acid samples from drug injecting environments would be important future work for identifying the risks posed by the use of these materials in this manner, as opposed to sterilised single use sachets.

Lemon juice samples

This was a separate exploration into the microflora of the lemon juice samples using the McLauchlin method. It would not be followed up by investigations into microflora within actual drug injections due to lack of available drug, however this would be suitable future work.

The lemon juice was investigated in respect to a number of reports (Gallo, Playfair, Gregory-Roberts, Grunstein, Clifton-Bligh, & Billson 1985;Mellinger, De Beauchamp, Gallien, Ingold, & Taboada 1982;Shankland, Richardson, & Dutton 1986) that indicated that the use of lemon juice to prepare injections was responsible for systemic, ophthalmic and other *Candida* infections. Investigations attempting to isolate fungal organisms from lemon juice

samples have previously been conducted, but this work differed from that through the use of a different method. Although the method uses many different types of media, the Sabouraud agar is specific to encourage the growth of fungi so was the most important media during this section of work.

Previous work (Collignon & Sorrell 1983;Shankland & Richardson 1989;Shankland, Richardson, & Dutton 1986) has suggested that the growth of fungi occurred within the lemon juice container after opening and use. This was suggested on the grounds that the preservative escapes from the container once the seal has been broken. Therefore the older the container, the more likely there was to be fungal growth within the juice (assuming that an organism had entered the container). For this work, newly purchased containers of lemon juice were compared with containers that were six months old (still within best before date). The old containers had been opened previously for particulate matter investigations, therefore they would have possibly been exposed to contamination.

During the initial phases of the investigation, it was clear that there were differences between the two different types of juice. The new juice was light yellow in colour, slightly cloudy, with a lemon odour. The old juice was orange-yellow in colour, heavily cloudy with a putrid smell.

As with the other samples, the two different types of lemon juice were prepared using the two parts of method 1. The spread plating of the first liquid samples from each of the lemon juice types resulted in no growth at all.

The second part of the sample treatment involving the filtration was impossible to conduct using the old lemon juice as it blocked the 0.2µm filter required in both instances. This was due to particles within the solution. Given that the filtration was incorporated into the procedure to remove the organisms from the drug solution to provide them with a drug free environment in which to grow, it was considered that it was less crucial during this drug free investigation. Additionally, the organisms of interest within the lemon juice samples were fungi, and these would be grown on the Sab agar from the initial samples.

The initial samples from both lemon juices produced no growth on any plate. This was the point at which fungal growth would be expected on the Sab plate. Although the old lemon juice was particularly cloudy and foul smelling, it appeared that none of these changes occurred due to bacterial action as none were found.

The samples of fresh lemon juice treated through the second sections of the produced growth on three plates from one sample- the method 2, unsteamed broth. These were not seen to be fungi, but bacterial rods. It is expected these were due to contamination of the samples.

The heroin powder

This was straight forward replication of the main work of McLauchlin. It was intended to provide a characterisation of the microflora present within the heroin samples provided by the police for this work.

The initial liquid samples produced no results. This demonstrated that either there were no viable organisms present within the drug samples, or that the drug present had an inhibitory effect upon the organisms present as has been suggested (Tuazon, Miller, & Shamsuddin 1980).

The broth samples produced the largest amount of results seen so far during the microbiology work. Growth was seen on 21 of 56 plates. It was clear that there were organisms present within the drug samples, and that the method successfully enabled their growth. This would enable the detection of these organisms from the prepared injections. If the organisms were able to survive the injection preparation method, then the results for the heroin injections would have been identical. Alternatively, the process might have affected certain organisms but not others, producing a different result to organisms seen from the drug itself.

Heroin injections

This investigation was conducted to a limited degree, but the results for this work were very promising, especially for the injecting drug user. There were no organisms detected from neither the initial samples, nor the incubated broths. Although one colony was seen, it was thought this occurred from external contamination, especially due to its location at the outer edge of the plate.

If repetition of this work produces the same results, from heroin with varied microflora, it would conclusive that the preparation of heroin in the manner demonstrated by the interview participants has beneficial effects in preventing the injection of many forms of bacterial and fungal contamination.

5.6.2 Limitations

Although these results are insightful, it would be unwise to draw any conclusions from the outcomes recorded here without further work, especially repetition of the work. The inability, primarily due to time constraints, to repeat the work at least three times makes the results inconclusive.

5.6.3 Method 2

This investigation had two distinct parts, firstly the investigation of the ability of organisms introduced to survive in a heated acid solution, and secondly the ability of the organism to survive a drug injection preparation.

B. cereus was seen to not grow on spread plates of any of the neat solutions. It was also not found within the broth solutions. This suggests that the preparation process of heating alone must inactivate it. Given that McLauchlin found *B. cereus* in 95% of heroin samples tested, this is very positive with regard to heroin injectors.

The *S. aureus* results were less promising. Growth was found in the spread plate of the lemon juice solution, and also in the broths from citric acid and lemon juice solutions. The growth from the citric acid was confirmed as *S. aureus*. The growth from the neat solution of lemon juice was seen to consist of two organisms one of which was possibility *S. aureus,* the other was a Gram variable rod. The growth from the broth was found to only consist of Gram negative rods. It would appear that this organism was introduced by the use of the lemon juice, given its ability to survive the injection preparation, combined with the possibility that at least one *S. aureus* appeared to survive within the neat solution, lemon juice use amongst injectors should be more actively discouraged than it is currently.

The injection preparation has a less acidic pH than the acid solution alone, but as has previously been demonstrated by Tuazon (Tuazon, Miller, & Shamsuddin 1980), heroin has antimicrobial activity. Whether this would lead to organisms being inactivated during the injection preparation was unclear. Organisms may have been inhibited by the heroin or the environment within the injection solution, but not killed by the process. Once injected into the body they would be free to proliferate given the right conditions, as they would be removed

from the injection environment. The first part of the this method assessed the affect the injection environment had upon the growth of these organisms, the second stage explored the growth of organisms that had survived the injection preparation out of the injection environment, hence postulating what organisms could proliferate after injection.

B. cereus was not seen to grow within the injection solution, suggesting it was inhibited by the environment. Spread plates of the broth suggested no growth from the citric acid solution, the ascorbic acid result being unclear. Streak plating of the broths produced growth results from both injections. Neither of the results were clearly *B. cereus*, but it indicates that at least two organisms, possibly three survived the injection process. In the absence of *B. cereus* it is likely the organisms were introduced within the drug sample.

The *S. aureus* samples produced growth from the neat ascorbic acid (none from the citric acid injection) and the broth samples showed clear growth of *S. aureus* on both the spread plates and the streak plates. Therefore it appears that it can survive the injection preparation. This has important implication for injectors. McLauchlin found *Staphylococcus sp.* in 40% of their heroin samples and Tuazon found them in 32% of samples, in addition they are very commonly found on the skin, so it is possible that they might transfer to injecting equipment when it is handled, for instance a spoon, and permeate an injection produced within it. This is of particular interest as Methicillin Resistant *Staphylococcus aureus* has been found in ulcers resulting from illicit injecting (Lettington 2002).

5.7 Pharmaceutics and Physical Characteristics of illicit drug injections

5.7.1 Pharmaceutical considerations of injection preparation

Parenteral administration of a substance into the body circumvents the natural defences of the body. It enables an injected product to come into direct contact with organs and tissues, which are protected from such interactions under all other circumstances.

The injection preparation is likely to present additional problems for the illicit drug injector. In addition to this, unsuitable substances and unsuitable injecting environment will be likely to compound issues. Factors such as insoluble particulate matter, or materials with extremes of pH when in solution, will lead to the preparation of a solution that may be dangerous to administer by the parenteral route.

All pharmaceutical products have to meet stringent criteria as set out in the corresponding pharmacopoeia such as the British or European Pharmacopoeia.

Aulton (Aulton 1988) suggests many factors that are important in the preparation of a parenteral product. Of these, the following appear to be the most relevant in the preparation of injections by drug users:

- The proposed route of the injection (IV/IM/SC)
- The volume to be injected
- The vehicle in which the drug is dissolved
- Particulate contamination
- The pH and osmolality of the injection solution

The route of administration

The injection route can vary; it is not limited to the intravenous route which is commonly used. Intravenous injectors with reduced venous access may resort to using the subcutaneous route. This can prove more dangerous than the iv route, as the damage caused by subcutaneous injection is much greater (Derricott, Preston, & Hunt 1999) and can lead to problems such as abscesses.

Subcutaneous and intramuscular injections carry the risk of infection by anaerobic organisms, which would not occur if the same preparation was used intravenously, including botulism (1995) and *Clostridium novyi* as discussed in section 5.1.2. Particulate matter will stay at the site of an extravascular injection over time, forming granuloma (Posner & Guill, III 1985).

Injection volume

The volume of injection solution is highly important. The volume to be injected tends to prescribe the route used- large volumes have to be administered intravenously, as they would be too painful subcutaneously or intramuscularly. To inject into muscle or subcutaneously, it is necessary to use a small volume which could be more concentrated, with a higher osmotic pressure, and therefore potentially more damaging. Injection of large volumes into an unsuitable site will increase the risk of physical damage to the tissue- injection of a large volume into a small vein, such as those in the feet, is a hazardous practice. In addition to this, the larger the injection volume, the longer the time the injection time will be, leading to more opportunity for damage to occur to the tissue being injected into, for instance, the longer the needle is in a vein the higher the risk of movement of the needle causing damage to the inside of the vein.

The common use of 1ml insulin syringes suggests many users tend to inject small volumes, however, injection of larger volumes will occur under certain circumstances, for example during the injection of solutions made from crushed tablets. Cyclizine tablets have a very low solubility and it is recommended that each tablet be dissolved in 1ml of water (Pearson, Gilman, & Traynor 1990). As users may inject ten to fifteen at once (ibid), this will result in an injection of a very high volume. Similarly, to discourage injection, oral methadone solutions are made relatively dilute. For any suitable dose of these to be injected, a large volume will have used.

Injection vehicle

The vehicle in which the drug is dissolved is important with regard to safety in terms of infection risk. Depending on its source, water may introduce contamination to an injection solution, for instance, dirty water, or water that has

been sitting in an open container. Distilled water or bottled mineral waters are potentially more hazardous than fresh water straight from the tap. Tap water has been chlorinated to kill micro-organisms to prevent diseases such as cholera.

Water for injections is the most suitable type of water, but as this is classified as a prescription only medicine (1968), it cannot easily be obtained for use, although June 2005 changes in legislation have its supply from needle exchanges (2005a). Harm reduction advice (Derricott, Preston, & Hunt 1999) recommends boiling water in a pan with a lid then leaving it to cool.

Sharing sources of water is another opportunity for infective organism transmission between injectors. Sharing can lead to the transmission of all major blood-borne viruses including Hepatitis B & C and HIV/AIDS, and it has been an important area in which harm reduction workers have focused in attempt to reduce these infections.

Pharmaceutics – Drug constituents

The preparation of any drug for injection introduces problems regard to its chemical form. Drugs found on the illicit market are rarely pure (Kaa 1994;King 1997;O'Neil & Pitts 1992). They are usually a combination of the drug, in varying concentrations depending on many factors such as which drug it is and its source, along with production by-products (impurities), various diluents that may have been added to increase the volume of drug, and general dirt/contamination that has mixed with the drug during its manufacture, transportation and storage.

The injection of oral preparations such as tablets and solutions pose particular hazards. Tablets have many excipients added to produce different pharmaceutical effects, such as slow release, or simply to hold the tablet together. These substances never normally enter the blood stream from oral ingestion of the tablet as they are insoluble, but when injected pose a high risk. Examples of such products include dicalcium phosphate (a diluent), microcrystalline cellulose (diluent), fumed/colloidal silica (a glidant), magnesium stearate (a lubricant), talc (lubricant) and stearic acid (lubricant)- at least one of these products is likely to be found in any given tablet, and they are all insoluble in water.

Commonly injected oral formulations include benzodiazepine tablets (Ross, Darke, & Hall 1997), sublingual buprenorphine tablets (Vidal-Trecan et al.

2003), Diconal® (a combination tablet containing an analgesic (dipipanone), and an anti-sickness drug (cyclizine)) and temazepam. The hazards of injecting Diconal were noted by Marjot (1978), who reported complications such as damage at the site of injection, including thrombosis and inflammation, vascular damage to lungs, lymphoedema, as well as deaths.

The injection of the hypnotic drug temazepam from liquid-filled capsules led to a spate of problems in the late 1980s. Its intravenous injection was first reported by Robertson et al. in 1987, and detailed by Stark et al. (1987), who noted that users had progressed from swallowing the liquid filled capsules, to extracting their contents with a syringe and injecting it. At the peak time of the message of HIV spread though heroin injecting, these temazepam injectors believed they were safe from infection as they were not using heroin (ibid). The liquid had a high viscosity, therefore a wide bore needle was required to draw it up and inject it. There were reports of users injecting up to ninety 20mg capsules per day (Farrell & Strang 1988) with many resulting complications such as abscesses and thrombophlebitis.

In an effort to cut down this abuse, the pharmaceutical companies reformulated the capsules to contain a thick gel (Gelthix®) instead of the liquid. This was done as it was impossible to draw the gel up into a syringe. Despite this, injectors of temazepam developed measures that enabled the gel to be injected. Most shook the gel in the syringe with boiling water, but other techniques used include heating it in a spoon, heating it with citric acid and vinegar, or using the alcohol from pre-injection swabs to liquefy it (Ruben & Morrison 1992). Injection of both liquid and gel filled capsules caused problems such as thrombosis and abscesses, and 25% of people interviewed by Ruben and Morrison who had injected the contents of gel-filled capsules had suffered a deep vein thrombosis. In an effort to use the thick solutions, the users turned to using large veins, such as the groin, to accommodate the large needles required to prevent blockage. This also led to accidental arterial injections, due to the anatomical proximity of these two femoral vessels. Once injected, the solubility of the gel decreased leading to it resolidifying after entering the vein. This effect resulted in serious complications such as compartment syndrome, rhabdomyolysis, deep vein thrombosis (DVT) and pulmonary embolisms. Treatments employed to treat the intra-arterial injections involved patients undergoing fasciotomies, amputations and haemodialysis (Scott et al. 1992).

Particulate contamination

In 1963, Garvan and Gunner published an article on extraneous particulate matter within injectable products. This was the first paper addressing the issue are particles within injectable products. This brought the matter to the attention of regulators and industry. Subsequent work investigated the effects of injection of particles of various sizes.

Standards for particulate content within injectable products were introduced into the British Pharmacopoeia in 1973.

The danger posed by particulate matter within injections is a matter of debate. The exact risks posed by injections containing small numbers of small particles are unclear, but injection large particles will clearly present more harm. The use of illicit injections is one of the main sources of information on the subject as drug injectors, especially those injecting crushed tablets, have enabled the observation of the results of high particulate injections.

Particulate matter injected into veins passes through the venous system, into the pulmonary artery to the lung where they will lodge in the arterioles or capillaries depending on their size. Smaller particles, less than approximately 8μm, will pass through the capillaries, back to the heart and then travel round the body via the arterial circulation. Depending on its size, the particles will lodge in a capillary vessel, or be ingested by circulating phagocytic cells or tissue phagocytes, such as the Kupffer cells within the liver.

Accidental injection into an artery is a serious event, especially if the injection solution contains a large amount of particulate contamination. Accidental injection into the femoral artery is commonly reported in illicit drug injectors who use the femoral vein. This leads to particulate matter obstructing the arterioles and capillaries down the leg, into the feet and toes. These blockages result in reduced blood flow, leading to widespread ischaemic damage, gangrene, deep vein thrombosis and may require amputation of the limb.

The effect of pH and osmolality of an injection upon veins

The pH and osmolality (Osmotic pressure) of an injection can be used to determine how irritant an injection will be upon administration (Kuwahara et al. 1998). The majority of work conducted in this area has investigated large

volume parenterals, particularly nutrition fluids. The effect these have is more profound as the blood vessels have extended contact with the solution due to the continuous flow. Smaller volume injections are expected to have less of an effect due to the rapid dilution of the solution once injected into a vein. The effect of injecting illicit drug solutions has shown that despite their small volume they are able to produce phlebitis, which can lead to thrombophlebitis (Derricott, Preston, & Hunt 1999;Scott & Bruce 1998).

Osmotic pressure is measured in units of milli-osmoles per litre (mOsmol/l). The osmotic pressure of plasma is within the range of 290-310mOsmo/l. It is recommended that any fluid with an osmotic pressure above 550 mOsmo/l should not be injected rapidly as this would increase venous damage and ideally injections and infusions should be between 300-500 mOsmo/l (Florence & Attwood 1998). Injections with a higher osmolality should ideally be administered very slowly to allow adequate time for dilution in blood.

5.7.2 Illicit injection investigations

To explore the risk posed by injection posed by illicit heroin injections, three areas of risk were assessed. These were the particulate content of the injections, the pH of the injections, and the osmolality of the injections.

5.8 Sub-visible particle investigations

5.8.1 Methodology

Previous work exploring particulate matter within illicit drug injections (Scott et al. 1997) used the electrical zone sensing (Coulter) principle. This process allows the determination of the size and number of particles in a solution. It achieves this by passing the solution through a small orifice that separates two electrodes, as the particles pass through the orifice, the change in resistance can be measured. This change in resistance allows the calculation of the particle size, and the number of resistance changes corresponds to the number of particles present.

The main drawback of this method is that the solution must conduct electrical current. If the sample does not conduct current sufficiently, it requires dilution with an electrolyte solution, usually sterile saline. This present two main problems; firstly the saline can affect the solubility of the contents of the sample, possibly leading to some precipitation of dissolved material, and secondly, the electrolyte itself adds particulate matter to the sample which needs to be subtracted from the sample result (background reading).

The British Pharmacopoeia used the Coulter principle as the method of particulate matter measurement until 1998. The method was superseded in the 1998 BP by methods based on light extinction. These methods involve the passing of the sample through a narrow glass tube illuminated by light on one side and a photodiode detector on the other. As the particles pass through the tube, they obscure the light, reducing the intensity of the beam reaching the photodiode. The change in the voltage flowing through the photodiode constitutes the size measurement. The number of measurements corresponds to the number of particles detected.

The primary advantage of this technique is that it involves no sample pre-treatment. The injection solutions can be analysed in exactly the form they would be administered, providing a true assessment of the particulate matter risk posed.

British Pharmacopoeia specifications

The BP (2000) contains three different tests for particulate matter within parenteral preparations. The first (Test A) applies to infusions or solutions for injection of volume over 100ml. The second (Test B) applies to infusions or solutions of less than 100ml. Test C applies to the testing of injections prepared from powders which are dissolved in a solvent immediately prior to injection.

Of these, the requirements for test C would be applied to the testing of the drug injection solutions. The conversion of the drug from powder to liquid injection form makes this appropriate test. This test has the highest allowances for particulate content.

Test limits

To perform the BP assay, four samples of not less than 5ml are analysed, with the results for the first sample being disregarded and the average for the remaining three being the test result.

A preparation complies with the test if the average number of particles present in the units tested does not exceed 10,000 per container equal or greater than 10μm, and does not exceed 1000 per container equal or greater than 25μm.

The contents of the 'container' in this situation were the 130mg sample of heroin (when used), and the other materials which are used to prepare a single injection.

A single injection will not produce 5ml for analysis. Pooling of solutions is used to increase the volume for the BP investigations, but this was not considered appropriate for this work. The time taken to produce each injection would result in cooling of solutions before mixing. This may have resulted in precipitation of the dissolved solids. Additionally the effect of mixing different injection solutions may have resulted in precipitation, for instance due to the common ion effect.

Considering a typical prepared injection resulted in a volume of less than 0.8ml, it was necessary to measure the quantity of particles in 0.1ml. In order to define test limits on this volume, it was chosen to equate the BP limit to that of a 0.1ml injection. Therefore, for 10μm or greater where the BP states the limit is 10000 per 5ml, this will be 200 particles per 0.1ml. For 25μm or greater, the limit is reduced to 20 per 0.1ml.

Illicit injections were not expected to conform to these limits, but the analyses would provide information on the particulate contamination of a typical injection and allow relative comparison with the limits placed on pharmaceuticals to present minimal 'risk'.

The environment in which the analysis is performed is also assessed by the BP method. Before any sample analysis is performed, particle free water should be examined to ensure that less than 25 particles of 10μm or above are measured in 25ml of the water. If the number exceeds this, the environment is contributing too much particulate contamination and conditions need to be altered to reduce this.

Apparatus

To perform these measurements, a Particle Measuring Systems Automated Parenteral Sampling System (APSS) 200 syringe sampling system was used. This system allows the measurement of particles in volumes of liquid from 0.1ml to 1000ml, and can size particles from 0.2µm to 125µm. The small sample volumes used by this machine make it particularly advantageous to the testing of the small volume injections that are produced by illicit drug injectors. The measurement of the particles in 0.1ml allows for the measurement of the number of particles in four portions (with the results obtained for the first portion being discarded as per the BP method) from one typical 0.8ml injection, without risk of drawing air bubbles through.

The APSS works by drawing the sample through a sample tube, to a detector, into a graduated syringe, then finally into a waste bottle. The syringe is motor driven by the machine and controls the sample volumes drawn into the sample tube. The system conforms to the BP (2000) specification, using light extinction as the method of particulate examination. For this purpose, the system uses a laser diode to produce a laser beam as the source of light.

The APSS system is controlled through a computer interface. This allows the configuration of every aspect of the machine. The results are displayed graphically as the machine conducts sample analysis and also during machine flushes, as a count of particles of certain sizes. The data is output in the form of a 'common separated values' (*.csv) computer file without the graphical data.

Channel data

The APSS-200 computer software allows the programming of 'channels' that can be adjusted to measure particles of different sizes. Each of the fifteen channels can be adjusted to count particles of a set size and above as far as the next channel setting. For instance, the detector can be set to measure 2µm particles and 3µm particles. However, the output data for the 2um channel will be a count of all particles between 2µm and 2.99µm in size. The 3µm channel count will cover particles 3µm and larger. Channels can be set to measure particles between 2µm to 125µm. Except for the validation (where channels chosen to best measure the known particle sizes were chosen), the channel

settings were kept at the default program settings to avoid each of the laboratory users having to change these settings between uses. As previously stated, the particles studied were limited to those outlined in the BP, that is particles of 10μm and greater, and 25μm and greater.

5.8.2 Materials

Standard equipment for injection preparation, including spoon, 1ml insulin syringes (B-D) and Swan® extra slim roll-up cigarette filters.

Screen wash (for cars, containing detergent, and ethanol/methanol) (Halfords)
Water for injection (Fresenius Kabi)

2ml sample (Eppendorf) tubes (Fisher)
Parafilm®
Stopclock

5.8.3 Methods

The APSS was prepared for use by flushing the system using the screen wash solution as recommended by the APSS manufacturers, followed by flushes of particle free water. The flushing of the system using WFI allows the user to ensure the machine is producing acceptable results before every sample run. The flushing of the WFI washes any residual particles out of the system, whilst the computer output confirms low particle counts.

5.8.4 Sample preparation

The samples for analysis were placed in new centrifuge tubes for use. Before use, each tube was rinsed three times using particle free water. The sample was then placed into the tube and the tube sealed. Gas bubbles in the solution were eliminated by allowing the solutions to stand for two minutes as per B.P. requirement.

5.8.5 Sample analysis

For analysis of all samples, the machine was fitted with a 1ml sample syringe, which enabled 0.1ml to 1.0ml sampling. The volume of the sample analysed depended upon the sample itself. Those in unlimited supply, for instance, WFI and the acid solutions, were analysed by the millilitre i.e. 1ml was the sample volume. For samples of limited volume, such as the prepared injection solutions, the sample volume was reduced to 0.1ml.

It is also important to note that the sample has to pass up a sampling tube before reaching the detector. This can be a problem when the volume of sample available is very small. The BP test stipulates that four measurements are taken from each sample, with the first result discarded, and the average of the remaining three calculated. To enable measurement of particles, the solution needs to be flowing through the detector during the time of measurement. However, given the internal volume of the sample tube, and the small volume of sample available, it would be possible for the measurement to be carried out on air instead of sample solution on the first and/or last samples.

To minimise the possibility of this, a custom modified sample tube was used, which was shorter than that usually used with the machine. The internal volume of the sample tube was approximately 0.4ml, therefore the machine required 4 additional sampling steps before the results could be recorded as the sample had yet to reach the detector. Once the method had been programmed into the computer it was found to work well, and produce repeatable results.

Before each sample was analysed, samples of WFI were run, and the output was checked to be within the range for a sample of WFI (less than 25 particles 10µm or greater in size per 25 millilitres of solution). Once this baseline was established, the machine was ready to analyse a sample. Before the sample was run, the machine was prepared by leaving a gap of air within the machine to prevent any mixing of the solutions within the sample tube prior to analysis.

When ready for analysis, each tube was opened and the sample tube inserted approximately 3mm from the bottom of the sample container. The open neck of the container was sealed using Parafilm® to prevent particulate contamination from the laboratory air, or the machine operator.

The sampling procedure was then initiated using the computer program. Once the sampling was complete, the machine was flushed using WFI. If the

sample under analysis produced large counts of particulates in WFI after several flushes, the machine would be rinsed using the screen wash solution. Further sample analysis would only be undertaken once WFI count levels returned to the acceptable limits described above.

5.8.6 Investigations

Firstly, to assess the likely background readings that would be obtained from the samples, particles in WFI were measured to establish limits on the numbers of particles that would be introduced into solutions when working with them, contributed by the operator and the environment. Particulate contribution by the equipment used during the processes was also assessed, including the syringes and eppendorf tubes, to measure their contribution to overall particulate count.

Secondly, particles present in WFI after drawing through various filters was measured to establish the effect the act of filtration had upon the particle content of injections. This was of interest as filters are used by injectors specifically for the purpose of removing particulate matter from their injections before use.

Thirdly, the first section of injection tests involved the testing of acid solutions without the use of drug. This provided information on the particulate contribution of the acids to the injection solutions.

Finally, the most important section of tests investigated the particles present in injections prepared in an identical matter to those of illicit injectors using illicit drug material.

5.8.7 Samples investigated

Standard solutions
(Samples analysed as 1ml of solution in order to obtain more accurate results, and as quantity was not an issue)

Water for Injection controls (WFI)

WFI from glass beaker (control)

WFI from rinsed centrifuge tube (control of sample container)
WFI from rinsed centrifuge tube via syringe and spoon (1ml drawn up into syringe, put into spoon, then redrawn up into syringe, then transferred to centrifuge tube) (control of injection procedure)

Filter solution controls

WFI drawn up into syringe from spoon through 1/3rd of Swan filter – through outer side
WFI drawn up into syringe from spoon through 1/3rd of Swan filter – through inner side
WFI drawn up into syringe from spoon through whole Swan filter
WFI drawn up into syringe from spoon through 2mm circle of Marlboro® filter (cut from the end of the filter, leaving the cigarette intact, but with a shorter filter)
WFI drawn up into syringe from spoon through cotton wool taken from a 'cotton bud'
WFI drawn up into syringe from spoon through a Sterifilter®

Acid solutions controls

75mg of citric acid in WFI heated in spoon unfiltered
75mg of citric acid in WFI heated in spoon filtered through 1/3rd Swan roll up filter (ripped side down, outer side)
170mg of ascorbic acid in WFI heated in spoon unfiltered
170mg of ascorbic acid in WFI heated in spoon filtered through 1/3rd Swan roll up filter (ripped side down)

Heroin injection investigations

130mg Heroin prepared with 70mg of citric acid, unfiltered
130mg Heroin prepared with 70mg of citric acid filtered with a Sterifilter®
130mg Heroin prepared with 70mg of citric acid filtered with a portion of cigarette filter
(The experimental work was conducted as part of a different investigation into particulate matter within injections depending on the use of different filters. The

work was conducted using 70mg of citric acid instead of the usual 75mg. Due to lack of drug the experiment could not be repeated using 75mg citric acid.)

Three solutions of each sample were analysed, each using the B.P. method of three measurements of each sample.

5.8.8 Validation

The APSS machine is calibrated yearly by the manufacturers, during this process, the machine is used to count the number of particles in a standard solution with known numbers of particles of known size. The results from the machine are used to produce a 'calibration file'. The calibration file is used by the computer software during the computation of the data received from the detector to give the true reading of particles within the sample. It is not possible for people other than service engineers to perform this calibration. The APSS was under valid calibration certification during the period of this work.

In order to validate the output of the machine, it was possible to ensure that the size measurement of the machine was correct. By using solutions containing latex beads of a known size it was possible to assess the output of the machine. The latex beads are purchased as a suspension in a small dropper bottle. To form a solution for validation, a small drop from the bottle was placed into 100ml of particle free water. The number of beads in one small drop was adequate for achieving high, clear readings from 1ml of the water.

During the manufacture of the standard solution the number of latex beads added to the diluting water cannot be controlled. Therefore, this method cannot be used to validate the number of particles counted by the machine. The size of the drop of bead solution used, the level of mixing of the bead solution in the dropper bottle (for instance, due to inadequate shaking), and the mixing of beads within the sample solution can all lead to different results.

Solutions of beads 5.0μm, 10.0μm and 16.7μm in size were available for use. As stated, solutions were made by dropping one drop of these suspensions into 100ml of particle free water. Samples of 1ml were analysed four times, with the first reading being discarded as per BP requirements. The size measurement channels were adjusted for the validation to measure the particles as accurately as possible given their known size. If the machine measured these particles as a different size, the accuracy of all of the measurements would be in

doubt. The channels of detector were adjusted from the standard settings to provide a good spread around the three particle sizes in use. The channels used were: 2, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 17, 20, 25µm.

5.9 Results

5.9.1 Validation

During validation of the APSS machine, it recorded the number of particles of each size as shown in the following graphs. Figure 20 shows the results for the 5.0µm bead solution, Figure 21 the results for the 10.0µm solution and Figure 22 the results for the 16.7µm solution.

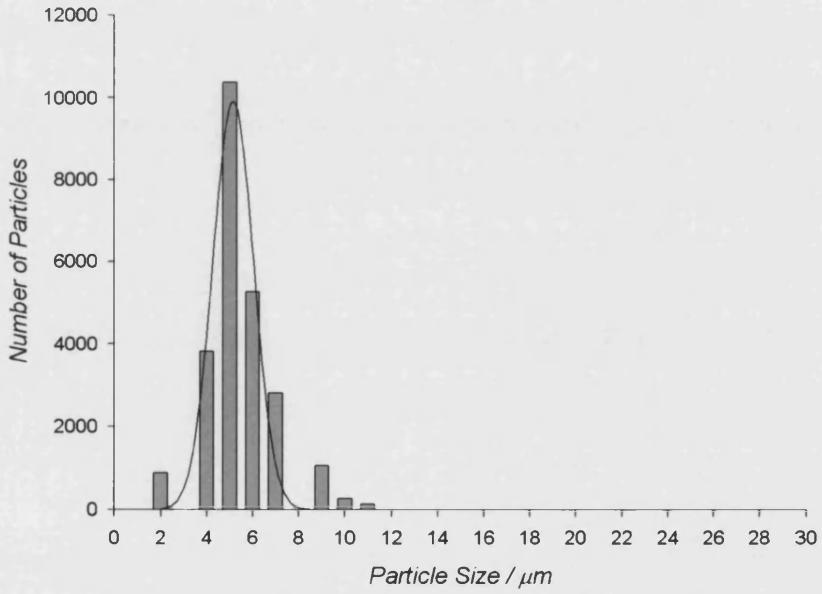


Figure 20 Particle distribution in 1ml samples of standard solution containing 5µm latex beads

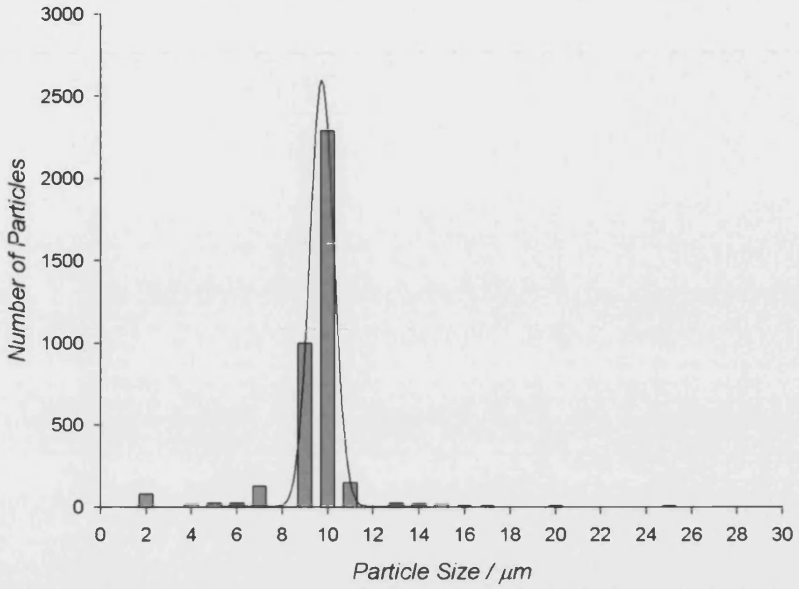


Figure 21 Particle distribution in 1ml samples of standard solution containing 10μm latex beads

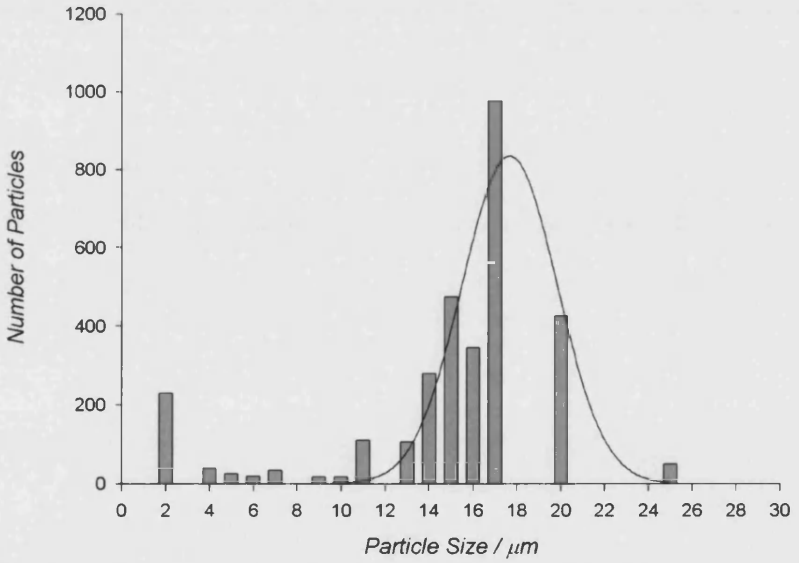


Figure 22 Particle distribution in 1ml samples of standard solution containing 16.7μm latex beads

The analysis of these solutions by the APSS-200 demonstrated that it was calibrated to measure the size of particles correctly.

These results were consistent through both the measurement of the different standards and consistent on a day to day basis.

5.9.2 Sample results

The following results are presented in three forms. Firstly, the tables within each section display the number of particles in the injection as specified by the BP (numbers above 10µm and above 25µm). Secondly, these results are displayed graphically. Thirdly, the final sets of tables show statistics relating to the 10µm (10.00 to 14.99µm) channel and the 25µm (25.00 to 29.99µm) channel.

5.9.3 Water for Injections investigations

WFI from beaker (control)
This served as the environmental control, in addition to the measurement of particles in water for injections. A 100ml glass beaker was washed three times with WFI, then filled with approximately 50ml of WFI and covered with parafilm. This was then analysed.

WFI from plastic eppendorf tube
An eppendorf tube was washed three times with WFI, then filled with 1ml of WFI which was sampled. This would establish the particulate contribution of the eppendorf tube to all samples analysed from these tubes.

WFI from spoon via syringe
An identical spoon to that which would be used to prepare injections was washed three times with particle free water. One millilitre of WFI was drawn up into a 1ml disposable syringe. This was gently pushed back out of the syringe into the bowl of the spoon, then gently drawn back up into the syringe. The water was then gently squirted into a WFI washed eppendorf tube for sampling.

Table 24 BP particulate limit results for WFI solutions

Particle size	WFI from beaker	WFI from plastic eppendorf tube	WFI from spoon via syringe
Over 10µm	0.067	0.778	3.444
Over 25µm	0.000	0.000	0.000

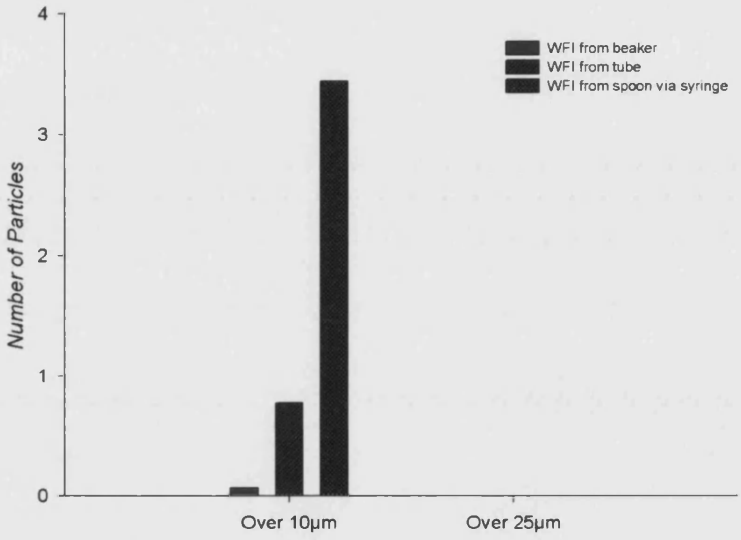


Figure 23 Number of particles in various WFI solutions

Table 25 Descriptive statistics for particulate content of WFI solutions

Sample	Particle size over:	Minimum	Maximum	Mean	Std. Deviation
WFI from beaker	10µm	0.00	0.00	0.00	0.00
	25µm	0.00	0.00	0.00	0.00
WFI from tube	10µm	0.00	1.33	0.556	0.694
	25µm	0.00	0.00	0.000	0.000
WFI from spoon via syringe	10µm	1.67	5.00	3.11	1.711
	25µm	0.00	0.00	0.000	0.000

The result for the WFI from the beaker was the confirmation that the environmental conditions for the particulate work were suitable. The result of

approximately 17 particles of 10µm or over per 25ml was well within the BP stipulated maximum of 25.

5.9.4 Water through filters

Seven different filters were used to filter WFI for analysis. For each of the following experiments, the tea spoon and eppendorf sample tubes used were rinsed three times with WFI prior to use.

WFI from spoon filtered through inner side of ripped Swan filter
One millilitre of WFI was drawn up into a syringe, and gently squirted into the bowl of a spoon. Approximately 1/3rd of a Swan roll up filter was ripped off a complete filter and placed ripped side up into the water. The syringe was then used to draw the water through the filter and back up into its barrel. This water was squirted into an eppendorf tube for analysis.

WFI from spoon filtered through outer side of ripped Swan filter
This was performed in exactly the same way as above except, the section of Swan roll up filter was placed ripped side down into the water.

WFI from spoon filtered through whole Swan filter
This was performed in exactly the same way as above except, a whole Swan roll up filter was used.

WFI filtered through 2mm slice of Marlboro 'light' filter
These samples were prepared using a 2mm thick circular slices cut from the end of cigarettes.

WFI filtered through cotton wool from cotton 'bud'
This sample was prepared using the cotton wool from the end of a Johnson & Johnson cotton bud.

WFI filtered through a Sterifilter®
This sample was prepared using a Sterifilter® from Exchange supplies.

Table 26 BP particulate limit results for WFI passed through various filters

Particle Size	WFI from beaker	WFI from spoon via syringe	WFI through Outer side Swan	WFI through Inner side Swan	WFI through Whole Swan	WFI through 2mm cig slice	WFI through Cotton Wool	WFI through Sterifilter
Over 10µm	0.067	3.444	1.800	0.600	236.600	5.533	19.933	21.000
Over 25µm	0.000	0.000	0.000	0.067	0.267	0.600	0.200	1.333

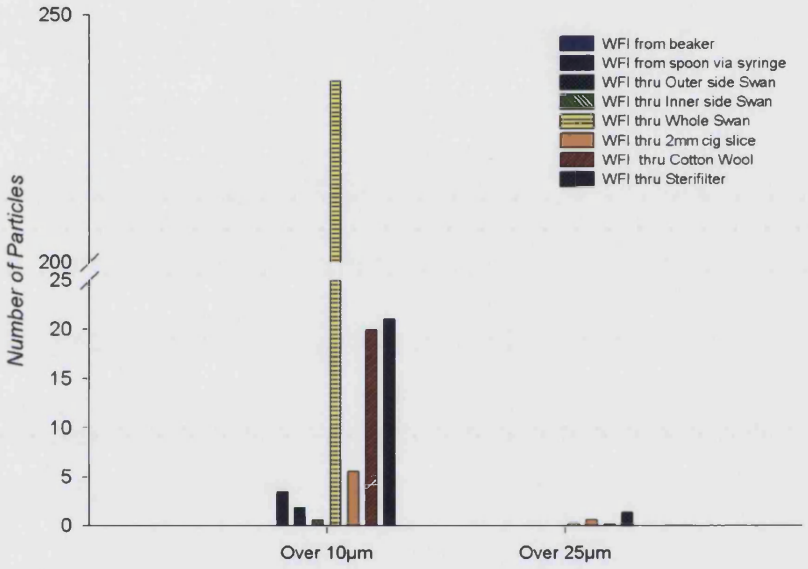


Figure 24 Number of particles in WFI filtered with various filters

Table 27 Descriptive statistics for particulate content of WFI passed through various filters

Sample	Particle size over:	Minimum	Maximum	Mean	Std. Deviation
WFI filtered through Inner side Swan	10µm	0.33	0.67	0.400	0.149
	25µm	0.00	0.00	0.000	0.000
WFI filtered through Outer side Swan	10µm	0.33	4.33	1.733	1.535
	25µm	0.00	0.00	0.000	.000
WFI filtered through Whole Swan	10µm	0.67	381.33	220.467	200.421
	25µm	0.00	0.67	0.200	0.298
WFI filtered through 2mm cig slice	10µm	0.67	9.67	4.267	3.345
	25µm	0.00	0.33	0.133	0.183
WFI filtered through Cotton Wool	10µm	1.67	34.33	16.800	15.668
	25µm	0.00	0.33	0.133	0.183

5.9.5 Acid solutions

Four different acid solutions were prepared for analysis:

Citric acid unfiltered

75mg of citric acid was placed into the spoon, 0.85ml of WFI was added using a syringe and the solution heated until the acid dissolved. The sample was drawn up into the syringe and transferred to an eppendorf tube for sampling.

Citric acid filtered

This was prepared as above, but the solution was drawn up from the spoon through 1/3rd of a Swan filter placed ripped side down, as witnessed in the injector interviews.

Ascorbic acid unfiltered and ascorbic acid filtered

These were prepared as above, but with 170mg ascorbic acid in place of the citric acid.

Table 28 BP particulate limit results for acid solutions

Particle Size	Citric Acid 75mg/0.85ml unfiltered	Citric Acid 75mg/0.85ml filtered	Ascorbic Acid 170mg/0.85ml unfiltered	Ascorbic Acid 170mg/0.85ml filtered
Over 10um	8.111	9.444	336.444	314.667
Over 25um	0.333	0.444	7.778	7.444

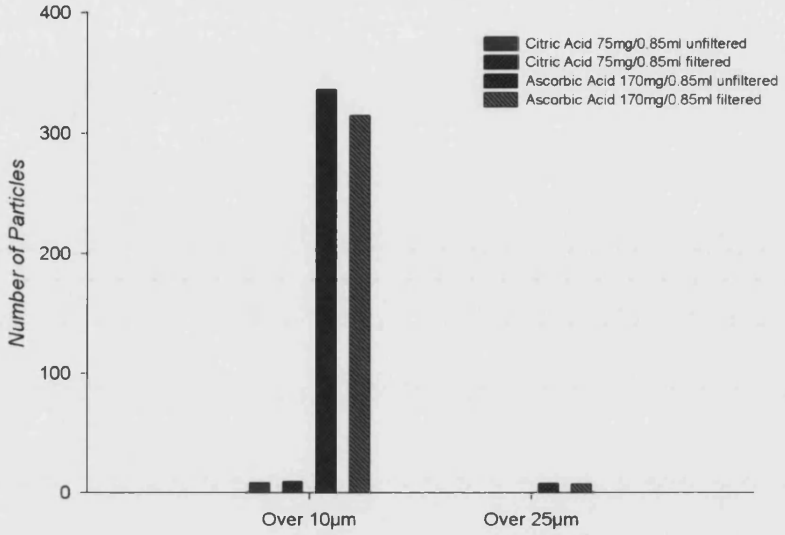


Figure 25 Number of particles in various acid solutions

Table 29 Descriptive statistics for particulate content of various acid solutions

Sample	Particle size over	Minimum	Maximum	Mean	Std. Deviation
Citric acid in WFI, unfiltered	10µm	4.67	10.33	6.778	3.097
	25µm	0.00	0.00	0.0000	0.000
Citric acid in WFI filtered with 1/3rd Swan filter	10µm	4.00	11.00	7.778	3.533
	25µm	0.00	0.33	0.111	0.192
Ascorbic acid in WFI, unfiltered	10µm	3.33	8.33	6.444	2.715
	25µm	0.00	0.00	0.000	0.000
Ascorbic acid in WFI, filtered with 1/3rd Swan filter	10µm	4.67	7.00	5.556	1.262
	25µm	0.00	0.67	0.333	0.333

5.9.6 *Drug injections*

The graph below depicts the spread of particulate matter found within three heroin injections prepared without filtration. This gives a baseline for the injections prepared using filters. The markers are spaced at the mean point of the channel range, and for this point it is important to note that the results placed at the 72.5µm range cover all particles measured above 30µm up to the APSS-200 maximum size of 125µm.

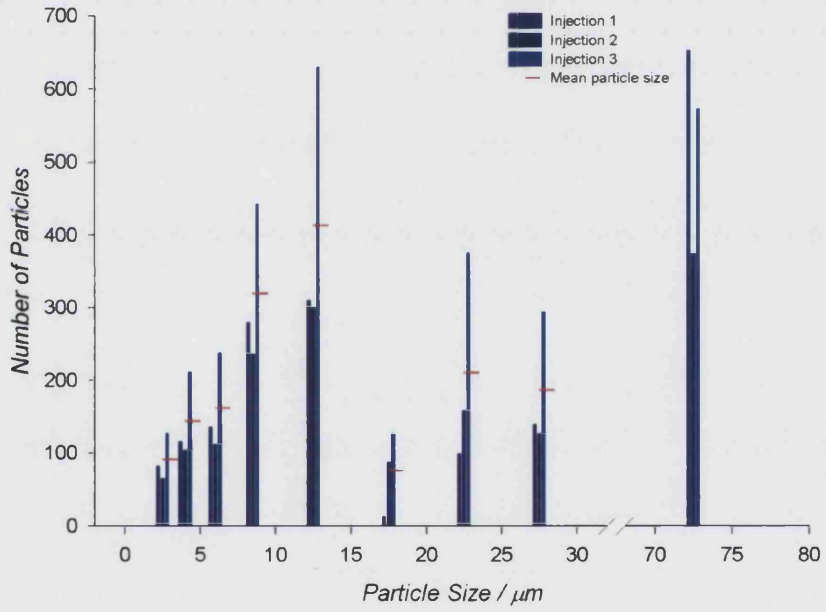


Figure 26 Distribution in size of particles in three unfiltered illicit heroin injections

Three illicit heroin injections were prepared for analysis. The first was unfiltered, the other two injections were prepared using two different filters.

Heroin prepared with WFI and 75mg of citric acid unfiltered
Heroin prepared with WFI and 75mg of citric acid filtered using 1/3rd Swan roll up cigarette filter
Heroin prepared with WFI and 75mg of citric acid filtered using a Sterifilter

Table 30 BP particulate limit results for heroin injections filtered with various filters

Particle Size	Heroin Unfiltered	Heroin Sterifilter	Heroin Swan filter
Over 10µm	700.000	276.667	1281.000
Over 25µm	719.000	12.333	234.333

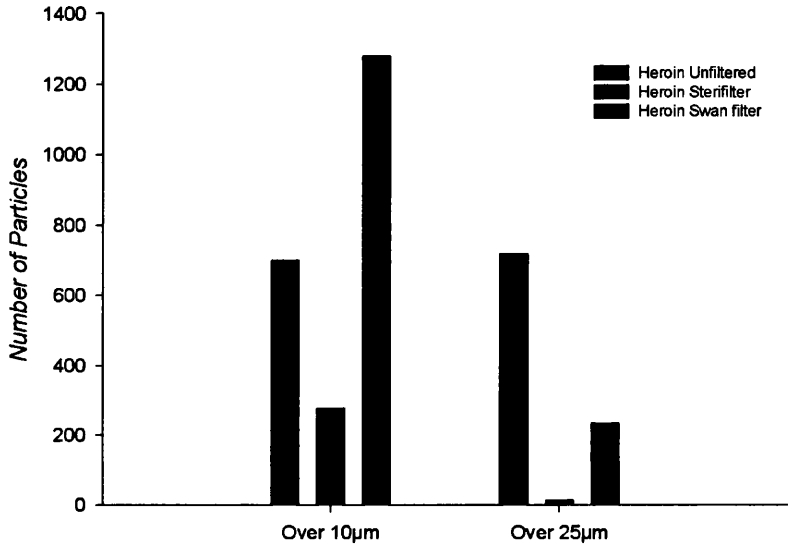


Figure 27 Number of particles in heroin injections when filtered using various filters

Table 31 Descriptive statistics for particulate content of illicit heroin injections prepared using different filters

Sample	Particle size over	Minimum	Maximum	Mean	Std. Deviation
Heroin Injection unfiltered	10µm	300.00	630.00	413.333	187.705
	25µm	126.00	294.00	186.333	93.468
Heroin injection filtered with Sterifilter®	10µm	153.00	249.00	205.3333	48.583
	25µm	3.00	8.00	5.6667	2.517
Heroin injection filtered with 1/3rd Swan filter	10µm	581.00	934.00	745.000	177.823
	25µm	111.00	150.00	129.667	19.553

5.10 Discussion

The initial measurements of the WFI from the beaker confirmed that the conditions under which the particulate analyses were conducted were suitable and would introduce minimal contamination into the samples. This was also important as it proved that the conditions under which the injections were produced (in the same laboratory) would not introduce contamination. The majority of particles measured within samples would have been introduced by the materials or equipment used in the preparation process.

The WFI sampled from the plastic sample tube showed an increase in particulate matter by approximately ten times that of the water from the glass beaker. This was most likely to have been the result of particles from the inside of the tube passing into the solution. The tube was washed with WFI three times prior to use as per BP specifications, but these particles remained, to pass into the solution. The particle counts were still within the BP limits.

The third manipulation of the WFI gave an indication of the particles introduced to the WFI from the spoon, the syringe and the exposure to the air during its movements. The results showed that of these three experiments, the WFI that had been placed into the spoon had the largest particulate counts. These counts were within the BP limits.

The use of filters led to a significant increase in the particulate content of the solutions. Although filters are used to remove particles from solutions, this is done at the expense of contributing some.

The Swan filter that was used by the majority of interviewees was tested for use in three manners. Although the use of a ripped filter with the ripped side up was considered to result in fibres being drawn up directly into the syringe, this was not borne out by the results. The use of the filter ripped side down resulted in three times as many particles 10μm or greater within the injection solution than the filter used ripped side up. The ripped side up filter did however show some particles above 25μm in side, the first detected in the investigations. From the raw data results it was seen these were above 30μm in size. The use of both filters still produced results within the BP limit for particulate content. It can also be seen that the numbers of 10μm particles are less than those recorded from WFI from a spoon unfiltered. It appears that they are acting to filter particles from the solution that have been contributed by the syringe, as the water is drawn up and particles from the spoon.

The use of a whole filter resulted in significantly increased particulate matter within the injection solution. For particles of 10μm or greater in size it exceeded to BP limits. This is postulated to have occurred due to the solution passing through more filter material, increasing the number of particles transferring into the solution. The number of particles within the solutions with the highest particle counts was significantly higher than the equivalent results for the ripped filters. Theoretically, the solution has to pass through 2 (3 thirds of filter) more layers of filter then with the ripped filters and would therefore be expected to contain approximately three times the amount of particles, but the measurements indicated significantly more than this. The reason for this is unclear.

The results from the use of a 2mm circle cut from the end of cigarettes showed an increase of the number of 10μm or greater particles over the use of WFI alone. Particles of 25μm or greater were also detected indicating these filters are both ineffective at filtering the solution and that they contribute to the overall particulate content.

The use of the cotton wool from a cotton bud was witnessed during the interviews by four participants. The use of cotton wool from this source raises questions about the possibility of the presence of glue used to bind the material to the plastic tube. This could possibly contribute particles to the solution, or dissolve into the injection solution. The results show a large number of 10μm or greater than the ripped Swan filters or WFI alone, but they were well within the BP limits.

The Sterifilter solution was found to contain a large amount of 10μm or greater particles as well having the highest counts for 25μm or greater particles than any of the other filters used. Both were within BP limits.

The investigation of the acid solution shows a difference related to the solubility of the acids. Citric acid which has the higher solubility of the two had low particulate counts, well within the BP limits, and the filtering of these solutions was seen to increase the particulate content due to the contribution from the filter material. The ascorbic acid had much higher counts for both levels of particle size, with the numbers of particles of 10μm or greater in both unfiltered and filtered solutions exceeding the BP limits. The filtering of the ascorbic acid solutions however did demonstrate that the use of the cigarette filter could reduce numbers of particles in both size ranges (over 10μm and over 25μm) despite the particulate contribution of the filter. The level of both particle

sizes in the filtered solution was less than that of the unfiltered solutions in contrast to that of the citric acid.

The results for the work with the heroin injections were the pinnacle of the work. The results for this demonstrated the effectiveness of the Sterifilter in respect to the use of cigarette material. The heroin injection solution that was unfiltered had a mean count of 700 particles for particles sized 10μm and greater, and 719 particles for particles of 25μm and greater. These are significantly higher than the BP limits, especially for the larger particle size.

The Sterifilter reduced the number of 10μm or greater particles by over half, although it was not able to reduce the number to meet the BP limit. Particles of 25μm or over were significantly reduced and to under the BP limit.

The use of the cigarette filter contributed to the number of particles 10μm or greater in size, therefore potentially posing more risk than using an unfiltered injection. However, it was useful in reducing the number of larger particles to approximately a third of the levels of an unfiltered heroin injection.

5.10.1 Filtration of injections discussion

The more manipulations a solution is put through, the more particles that are found in the solution, due to particles entering the solution from the surfaces it comes into contact with and from the air. This is demonstrated by the first set of water measurements, however, for illicit injectors, these factors are almost irrelevant as it would be impossible to reduce them. Injection preparation within a particle free environment as found in the pharmaceutical industry is not an option or consideration. Dirty equipment and environments are likely to add to the numbers of particles found within injections.

The use of filters was seen to add particles to the injection solutions. Filters should ideally remove particles from solutions, without adding particles of their own. It is clear none of the filters used by injectors are ideal; however, the most commonly used filters- ripped sections of Swan filters and slices of cigarette filter- contributed lower numbers of particles than the alternatives.

The use of a whole Swan filter demonstrated that the more of the Swan filter material the liquid passes through, the more particles that are shed into the solution. The use by injectors of a whole filter was found to be uncommon during

the interviews, probably due to its level of fluid retention. This may prove to be beneficial for injectors, as they will be unaware of its particulate contribution.

Cotton bud cotton wool was expected to introduce particles. The method of pulling the fibres off the rod with the fingers and rolling them into a ball were expected to add particles, and it is probable that there is glue present on the fibres that may pass into the solution. The high number of particles measured in the test solutions was expected. The use of cotton wool by injectors is already discouraged and this work demonstrates this is valid advice.

The testing of the Sterifilter® revealed that it does appear to shed particles into the injection solution, but its effect of filtering out the particles from the heroin injections proved to be highly superior to the filtering effect of cigarette material.

When the number of particles contributed by a filter to the injection solution is compared to those present within a prepared injection solution it is clear that they are a very small proportion. Therefore, the shedding of the particles is of less importance than the ability of the filter to remove particles within the solution.

The Sterifilter supplied as a sterilised package for single use is the best filter to recommend IDUs to use. Although it was unable to enable injections to pass the BP limit for particles of 10μm or greater, it made a significant reduction on the numbers present within an injection. More importantly it reduced the number of larger particles to approximately half the BP limit, and as these particles are more likely to lead to complications than smaller particles this is a crucial point. Combined with the sterility of this product, it is the ideal choice for use.

5.11 Laboratory investigations of injection pH

5.11.1 Introduction

During the interview stage of this project, the interviewees were found to use three different acidifying agents, namely citric acid, ascorbic acid and lemon juice. During the preparation, a range of quantities of these acidifiers were used.

The pH of the injection solution will relate to the solubility of the drug, by using different concentration of acids in solution, the pH will vary, therefore the amount of drug in the solution will vary. The pH of the solution is also of importance with regard to the potential the solution has to cause to damage the tissue lining the blood vessels into which it is injected. Once injected into the bloodstream, the buffering capacity of blood and the dilution of the solution will change the pH to that of the blood.

This experiment intends to measure the pH of solutions covering the range of solution strengths that were seen in the interviews.

5.11.2 Materials and equipment

Materials:

Ascorbic Acid	(Sigma)
Citric Acid	(Sigma)
Lemon Juice	(Jif®)
Distilled Water	(Milli-Q)
Tap Water	(Wessex Water)

Equipment:

Balance	(Mettler AE 50)
pH Meter	(Hanna – pH 302, with Hanna probe HI 1330B (slim probe for use in small eppendorf tubes containing low volumes of sample))
Temperature probe	(Hanna – pH 302)
Automatic pipettes	(Gilson®)
Sample tubes	(Fisher)

5.11.3 Method

The interview work demonstrated use of ascorbic acid and water quantities that could produce solutions from 40mg/ml up to 300mg/ml. For citric acid, the solutions ranged from 16mg/ml up to 405mg/ml. (The 405mg/ml was

an exceptionally high value, with only two users employing solutions over 300mg/ml). A range of solutions falling between these values were made up, and the pH and temperature measured.

Dilutions using lemon juice were made up in the same proportions as witnessed in the interviews. To account for variation in samples, the juice from three different Jif® lemons was tested.

The solutions were made up using distilled water and tap water drawn from the cold water tap in the laboratory. All solutions were made using temperature at room temperature, approximately 25°C.

The pH of six heroin injections was investigated. The details and reasoning regarding the acids quantities used to prepare these was discussed in section 3.6.3.

5.12 Results

Distilled water used pH 5.41 at 25.6°C
Tap water used pH 7.76 at 25.3°C

Citric acid

The citric acid solids dissolved easily in both waters with a little shaking.

Table 32 pH of citric acid solutions made with distilled water and tap water

Solution	Distilled Water		Tap Water	
	pH	Temperature/ °C	pH	Temperature/ °C
15mg/ml	2.14	23.2	2.27	23.6
100mg/ml	1.74	23.4	1.78	23.6
200mg/ml	1.61	23.2	1.55	23.7
300mg/ml	1.44	23.4	1.48	23.5
400mg/ml	1.35	23.2	1.38	23.5

Ascorbic acid

The solutions of ascorbic acid proved hard to produce at concentrations above 150mg/ml as the solid acid particles required the solution to be shaken for a long time to allow them to dissolve.

Table 33 pH of ascorbic acid solutions made with distilled water and tap water

Solution	Distilled Water		Tap Water	
	pH	Temperature/ °C	pH	Temperature/ °C
40mg/ml	2.49	25.3	2.71	25.9
100mg/ml	2.24	25.2	2.42	25.6
150mg/ml	2.17	25.3	2.26	25.9
225mg/ml	2.03	26.0	2.10	26.6
300mg/ml	1.91	27.7	1.96	27.2

Lemon juice

Undiluted lemon juice

Lemon 1 pH 2.46

Lemon 2 pH 2.49

Lemon 3 pH 2.50

Diluted lemon juice results

Table 34 shows the pH of lemon juice solutions when diluted with distilled water and tap water.

Table 34 pH of lemon juice diluted as demonstrated by drug injectors

	Distilled Water		Tap Water	
	pH	Temperature/ °C	pH	Temperature/ °C
Lemon 1				
0.3ml LJ in 1.2ml Water	2.62	25.2	2.74	25.2
0.4ml LJ in 0.8ml Water	2.57	25.6	2.62	25.4
Lemon 2				
0.3ml LJ in 1.2ml Water	2.63	25.3	2.69	25.5
0.4ml LJ in 0.8ml Water	2.60	25.2	2.60	25.7
Lemon 3				
0.3ml LJ in 1.2ml Water	2.63	25.4	2.74	25.5
0.4ml LJ in 0.8ml Water	2.61	25.6	2.65	25.7

5.12.1 pH measurements of drug injections

Injections containing heroin were produced using the standardised preparation technique, including 130mg of heroin powder. Due to limited supplies of heroin at the time of production, the preparation of injections was limited to variation of three different quantities of citric acid, and three different quantities of ascorbic acid. Each was prepared using 0.85ml of water as standard. All were produced in duplicate to confirm results.

Injections prepared:

Citric acid
50mg
70mg
140mg

Ascorbic Acid
135mg
175mg
330mg

Initially, lower quantities of each of the acids were attempted for use- 25mg of citric, and 90mg of ascorbic. These were exactly half the mean quantities defined in the standard procedure. These quantities of acid were insufficient and the drug was clearly did not dissolve. Figure 28 below shows an injection mixture prior to heating; solids are clearly visible. Figure 29 was taken after an injection solution had been drawn up into the syringe. Dissolution had appeared to have taken place when the solution was drawn up, yet sediment clearly remained at the bottom of the spoon. This would most likely be unacceptable to any drug user, who would probably make further attempts to dissolve the powder.

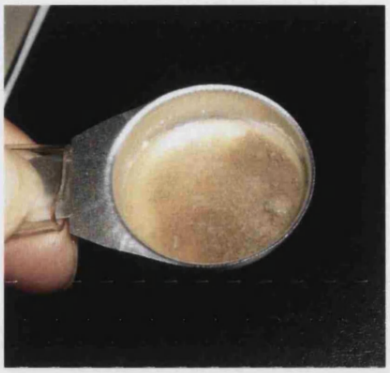


Figure 28 Heroin powder in mixture prior to heating

Figure 29 Undissolved drug material left in the cooker

The step was taken to add extra quantities of acid until the drug was seen to dissolve.

5.12.2 pH measurements of prepared heroin injections

The following tables display the pH of the prepared heroin injections. The measurements were taken directly after the preparation of the injection. All readings were taken at approximately 25°C.

Table 35. pH readings for injections prepared with citric acid

Citric acid quantity	pH		
	Injection 1	Injection 2	Average
50mg	2.96	2.96	2.96
70mg	2.63	2.60	2.62
140mg	1.85	1.83	1.84

Table 36. pH readings for injections prepared with ascorbic acid

Ascorbic Acid quantity	pH		
	Injection 1	Injection 2	Average
135mg	3.30	3.22	3.26
175mg	2.93	2.97	2.95
330mg	2.40	2.35	2.38

5.13 Discussion

The results from this section of the project are of importance as they give an insight into the effect the solutions will have the body tissues it comes into contact with upon injection.

The results from the measurement of pH of acid solutions are unsurprising; the stronger the acid solution, the lower the pH. Additionally, as the temperature of the solution is raised, so the pH lowers. These results were important for comparison with injection solutions containing drug.

When drug was used to prepare the injections it was seen that the pH of the lower strength acid solutions was higher than that of the acid solution of the same strength alone. For example, citric acid 100mg/ml solution alone was pH 1.74, but with drug the measurement is raised to 2.64 (88mg/ml citric acid actually used). The results were similar for the ascorbic acid, where the 150mg/ml acid solution had a pH of 2.26, but a heroin injection containing 158mg/ml ascorbic acid had a pH of 2.93.

As the amount of either acid is increased in the injection solution, the pH draws closer to that of the acid solution alone. This demonstrates that injectors choosing to use large quantities of acid are at potentially at risk of pain, and possibly further complications, from the low pH of the injection solutions.

This concurs with current harm reduction advice, and as previous work has demonstrated (Scott, Winfield, Kennedy, & Bond 2000) there will be an optimal amount of acid required when producing an injection. The addition of further acid will dissolve little or no additional drug. The difficulty for the injector is gauging the optimum quantity to use, as there is no simple way to determine it, especially given the variability of illicit drug mixtures.

5.14 Osmolality of prepared heroin injections

Osmolality was measured using the freezing point depression technique, using supercooling. This is the British Pharmacopoeia method (2000). To perform this, an osmometer is used. This was done using the Advanced Instruments Micro-Osmometer model 3300. The analysis performed by Dr. Richard Headford, at the pathology laboratory of the Royal United Hospital in Bath, as the facilities were not available at the University.

The osmometer requires a minimum of one millilitre of sample for analysis. To enable the measurement of illicit injections, two identical injections were prepared using each acid quantity. The resulting injections were then pooled together to produce the sample for osmolality analysis. This pooling was necessary for the osmolality measurement conduction and was considered to not adversely affect the samples results, for instance through any decrease in solubility, or precipitation of the dissolved materials within the samples.

The samples investigated were the same injections prepared for the pH investigation, above. Each sample was analysed twice.

5.15 Results

The following tables depict the results for the osmolality measurements of prepared injection solutions. The solutions analysed were those prepared for the pH measurements above.

Table 37. Osmolality readings for injections prepared with citric acid

Citric acid quantity	Osmolality (mOsmols)		
	Injection 1	Injection 2	Average
50mg	493	490	492
70mg	525	533	529
140mg	1001	1011	1006

Table 38. Osmolality readings for injections prepared with ascorbic acid

Ascorbic acid quantity	Osmolality (mOsmols)		
	Injection 1	Injection 2	Average
135mg	1292	1266	1279
175mg	1559	1623	1591
330mg	n/a*	n/a*	-

*No result was recorded for the 300mg ascorbic acid injection as the solution would not freeze, despite repeated attempts

5.16 Discussion

The osmolality investigation results indicate that injections prepared using 50mg of citric acid, and possibly those using 70mg, are unlikely to cause problems when injected as the osmolality is below 550mOsmo/l. Those prepared using quantities of citric above this, and any quantities of ascorbic acid will exceed this 550mOsmol/l, leading to potential complications.

As the results demonstrated, the lowest quantity of ascorbic acid to suitably dissolve the heroin was 135mg. Indeed as the interviews demonstrated, the mean quantity used was 175mg. The injection produced using 135mg had a mean osmolality measurement of 1279mOsmol/l , which is over twice the recommended 550mOsmol/l. whereas the 175mg injection gave an even higher osmolality reading, a mean reading of 1591mOsmol/l.

Therefore, any injection prepared using ascorbic acid would be expected to cause complications upon injection. The interviews contradicted this. Participants claimed to prefer ascorbic instead of citric on the basis they considered it to be a safer acid. Additionally the burning upon injection of injections prepared with citric reinforced these feelings.

These results suggest that it is the pH of the injection solution that is related to the burning sensation upon injection. This does not mean that the ascorbic acid injections do not cause damage to the blood vessels as the osmolality results suggest it should. Further work is required to determine the effect these solution have upon the tissues they come into contact with upon injection.

6 General Discussion

This research project has covered a range of investigations, in areas that have little, or no, previous work. Investigation of the techniques used to prepare injections, and then their translation into a suitable laboratory method is a novel concept. Work of this nature is arguably long overdue. The prevalence of illicit injecting reinforces the need for such research.

The investigation of injection preparation, and the resulting injections are akin to the work conducted by Huizer (1987) on the techniques and outcomes of smoking heroin. Huizer's research was conducted during the late 1970s and early 1980s. The fact that this work was conducted over twenty-five years ago, makes it surprising that equivalent work into injections has not been previously conducted. Advances in laboratory techniques, combined with changes in the constituents of street drugs and changes in smoking techniques would suggest that the smoking research is a suitable area for further research again.

6.1 Methodological Critique

6.1.1 Interviews

The drug injector interviews were designed to explore the manner in which drug users prepared drug injections, and then to explore these preparation methods in detail, so a lab technique could be based on this information.

In practice the interviews were conducted without problems, and recruitment for them proceeded quickly. Interviews at needle exchanges provided a large number of current injectors, many of whom showed willingness to participate in the research. Additionally, no participants withdrew during any of the interviews.

Recruitment for the pilot interviews in the Bath exchange was low; it is possible that the lack of compensation for the time spent during the interview was a factor. Although potential interviewees were not informed of payment prior to interview, the possibility that they had been told of the payment by previous

participants was considered. This may account for the greater willingness for participation in the two areas where payment was made.

The interviews were conducted in preference to focus groups as discussed in the methodology section of the interview work chapter. The outcome of the interviews indicates they were a suitable choice for the investigation of the preparation process. Focus groups would not have provided the vital quantitative data. In addition to this, whether the recruitment of people to partake in focus groups which would last significantly longer than the interviews is unclear, but it would be expected to have been more difficult with regards to arranging attendance. The use of these interviews is considered to have been the best choice, and they proved to be a viable and productive method.

The questionnaire section of the interviews explored a range of subjects, from participant demographics, drug history, injecting history and injection preparation. This enabled a wealth of data to be recorded, although on reflection some was subsequently deemed superfluous to the overall object of the project work. Data covering areas such as drugs used, injection sites, and geographical variations were beyond the needs for the design of the standardised injection for the laboratory work. These data will however, prove useful for future publications.

The injection preparation demonstration was designed to record the preparation steps and the materials and equipment used by the interview participants. They achieved this aim, recording a high level of detail of each procedure demonstrated. Searches of the literature were unable to find any non-participant observation studies conducted within this field previous to this work.

The recording technique proved capable of documenting the process as it was performed. All details of the procedure were recorded, allowing accurate replication of the procedure within the laboratory setting.

Although the objectives of the interviews were met by the design, problems were evident. For instance, there was a recurrent problem with the timing the heating of the injection solutions. The stopwatch was started when the heating began, but the fast paced nature of the recording sometimes resulted in the watch not being stopped as the participant quickly moved onto the next stage of the process. When this occurred, no heating time was recorded for that interview.

To improve the interview process, the use of additional recording methods would increase the accuracy of the data. Although considered, but

rejected during the interview design phase, the inclusion of audio or video recording could have been beneficial. Audio recording could improve accuracy of data especially during the questionnaire phase. Video recording would be of benefit through the entirety of interview, especially during that of the demonstration section. The use of video was rejected primarily on the grounds of confidentiality concerns, although non-participant observational studies conducted subsequently by (Taylor et al. 2004) have shown the superiority of this technique with regards the richness of the data recorded. This study, conducted in Scotland, addressed the confidentiality of this type of work and demonstrated that the steps can be taken to protect the participants, even enough to allow delegate viewing of the footage at conference events. Using the same procedures as Taylor et al. would be likely to satisfy ethics committee requirements for the use of this method in future preparation method investigations. Experience gained during this project would possibly endear ethics committees to grant approval for in-depth interviews utilising video recording and non-participant recording of the preparation process as further work.

The wording of some of the questions regarding drugs and acids ever used were possibly of a risky nature. It is possible that some participants had never known such drugs were injectable, or had never considered the use of some of the more questionable acidifiers, such as descalers. A risk of promoting risky behaviour was therefore present, which could pose ethical issues. The inclusion of these substances in the probes list was not ideal, however they were valid for the majority of participants. The decision during the interview whether or not to ask about the use of these substances, based on a combination of factors including, the injectors age, length of time injecting, drugs stated used and on a general feel for the overall experience of the participant. Due to the informal setup of the interview rooms, it was possible for the participants to read the options available on the list of probes, therefore, the riskier probes were written in strike through font, for instance ~~buprenorphine~~ in an effort to reduce the participant's ability to read this word upside down from the interviewer's sheet.

The decision of whether to suggest the probes to some interviewees, whilst withholding them from others, was questionable practice. Bad judgement could have resulted in interviewees unsuitable being prompted inappropriately as was trying to be avoided. This judgement by the interviewer also potentially

introduced a level of interviewer bias. Poor judgement may have resulted in participants who were suitable for probes not having then suggested. Despite these issues, it was difficult to address the issue in a different manner. The probes were required as without probing for further details, the participants may have forgotten to mention these drugs/acidifiers. Not using these probes would have adversely affected the quality of results. Removal of some of the more risky substances from these lists could have been possible, but to the detriment of the overall results.

Data analysis could have been improved through the use of SPSS instead of Microsoft Access, but during the initial data input it was considered that Access would give better data handling facilities. In consideration, the input of the data into SPSS in the first instance would have resulted in a better ability with which to handle the coded data, as well as saving time and effort which was subsequently used to copy data from Access into SPSS for statistical and graphical analysis.

6.1.2 Extrapolation of the amount of drug used

The methodology section of the interviews describes the reasoning for the use of 'fake' drug during the interviews, instead of the use of real drug. The observation of injectors preparing real drugs conducted by Taylor addressed the ethical and legal implications of the presence of non-participants within the drug injection environment. This was one major concern for this project as it would have been the first work to have incorporated the use of illicit drug material during interviews. It was considered too risqué for the required outcome of the interviews. The work of Taylor did not address the injection preparation in as much detail as was required for this project, it was concerned primarily with identifying risky behaviours exhibited by injectors, particularly those of surrounding blood-borne virus transmission. Quantities of materials used were not measured, and this was critical data required for this project to allow reproduction in the laboratory.

To measure quantities of drugs used, two different approaches were considered. Either the interviewer would provide a sample of drug of known quantity (as done during this work with the fake drug), or the interviewer disturbs the participants during the preparation to measure quantities in use. The first method would have legal implications and could put the interviewer at personal

risk. The second method would be hard to conduct as it would involve stopping the participants during the preparation to measure the quantities. They may be unhappy about this, and it would be likely to disrupt the flow of their preparation, which then may be altered to accommodate the interruption.

After the interviews were conducted, the requirement of having to use fake drug materials introduced the complication of converting the amount of 'fake' powder used into that of real drug material. The conversion attempts made in the laboratory are not ideal, but were the best conceivable. They rely on the visual nature of the measurement of drug quantity. This is a crude conversion and one that is very subjective- one person's measurement may vary from that of another.

The ideal method of comparing these two powders would be to get the users to measure both out in a similar manner during an interview, however, with access to real drug powder, it would make little sense to have to employ a 'fake' drug. Until interviews can be conducted using real drug powder available for measurement these results are all that is currently available.

6.1.3 Laboratory Investigations

The basis of this work was to investigate the pharmaceutical properties of the injections prepared in the manner demonstrated by the interview participants. The work did not aim to pursue method development, but rather to use existing methods applying them to the area of study.

Drug content of prepared illicit drug injections

The first laboratory investigation undertaken was measurement of the level of drug contained within each injection. This was expected to vary depending on the quantity of acid used to prepare the injection, and possibly due to additional other factors which could have been further investigated.

Prior to the commencement of this work, the method using capillary electrophoresis as used by Taylor (1996), and subsequently Scott (2000), was the intended process by which to perform this measurement. The outlined problems with equipment required an unexpected and undesirable change of technique. Alternative methods of measurement of heroin within illicit drug

samples were investigated, but these are either were unavailable due to lack of access to specialised equipment, or not suitable such as the normal phase HPLC method of O'Neil and Pitts (1992). These were other reasons that CZE had been selected for use in the first place.

To conduct these measurements, a new method had to be found and developed. The intention was to perform as little manipulation of the injection solution before analysis, so as to give as accurate measurement as possible. Ideally, a measurements performed on neat injection solution would be ideal but no current method will allow this. The use of CZE would require the sample to be diluted with methanol before analysis. Other methods require even further treatments, such as gas chromatography, which would require removal of all water from the samples followed by dilution within an organic solvent for analysis.

It is possible that a modification of the electrospray mass spectrometry method, as used to analyse the drug powder samples, would enable quantification. There are publications that prove this method can be used effectively (Selby 1998). Equipment availability problems prevented this work being conducted, although it is potential work for the future.

The use of NMR for quantification of the amount of drug within the samples was the application of a novel, and relatively uncommon use of NMR. This method was chosen for the access to the NMR equipment, and after the realisation that NMR could possibly be used for quantitative measurement. The tentative use of this method proved it was possible, but the necessity to prepare the injections through the process of freeze drying, followed by dissolution in methanol-*d*3 was additional manipulation of the samples, which may have adversely affected them producing misrepresentative results. Additionally, once freeze dried, samples could have been analysed through a number of other means, for instance, gas chromatography using methods such as that of Kaa (Kaa & Bent 1986).

Microbiological methods

The microbiology methods used proved suitable for the study of the injections. The use of the McLauchlin method (McLauchlin, Mithani, Bolton, Nichols, Bellis, Syed, Thomson, & Ashton 2002) was chosen over that of the BP sterility test (2000) on the basis that it would allow identification of organisms that were cultured from the samples. The BP sterility test would also be less likely to grow fastidious organisms especially the *Clostridia* that the McLauchlin method incorporated specific media for. The McLauchlin method was devised in response to the outbreak of *Clostridium novyi* that was responsible for the deaths of illicit heroin injectors. It was developed primarily to identify the existence of these bacteria within drug samples, but the design of the whole method allowed the isolation and identification of a wide range of different bacteria and fungi.

The second microbiology method used was not a published method. It was designed during the controls stages of the first method. The acquisition of cultures from the university culture collection allowed the inoculation of drug samples with known organisms to investigate the ability of these organisms to survive the preparation process. Compared to the method of McLauchlin this method was much less intensive with regards materials and time.

The main limitation of this section of work was the fact that the drug samples had already been shown by method one to contain viable organisms. This could have resulted in these organisms surviving the preparation process and growing as the dominant organism within the broth. The possibility of sterilising the drug samples was not an option as a process such as autoclaving, or dry heat sterilisation would potentially adversely affect the drug samples. Any growth resulting from the samples would be proof that at least one organism was able to survive the preparation process, even if it was not the introduced organism.

General Microbiological Investigation Problems

Two main issues were raised during this area of work. The first involved the use of micro-organisms with specific handling requirements. Recent

changes in handling procedures and regulations led to delays in clarification with safety officers over the possible use of certain organisms, notably *Clostridium botulinum*, which the McLauchlin method required as a control. The use of this organism was ruled out by the Pharmacy department after a delay, even though the use of a technique that would specifically culture the organism, had it been present within the illicit samples, was authorised.

The second issue was that of organism identification. During the planning phases, the identification of any cultured organisms down to species level was envisaged. Although this is possible using equipment such as API test kits, it was found to be prohibitively expensive. Further consideration of the McLauchlin paper makes it apparent how difficult this identification is, even for a specialist microbiology unit such as the PHLS. Their main work was conducted in a number of locations including the Public Health Laboratory Service (PHLS) Food Safety Microbiology Laboratory in North London. Limited identification could be conducted there, and specialist identification was required for the anaerobes and *Staphylococcus sp.* produced. These included sending cultures to the PHLS Anaerobe reference unit in Cardiff, and the Laboratory of Hospital Infection (CPHL) in Colindale, London. Third party identification would have been possible for this project, but again was beyond its financial scope.

Further to these issues, the amount of microbiological work conducted was limited by the cycle time required for the conduction of one set of experiments. The microbiology experiments were one of the latter stages of work and this was necessary due to the requirements for the injection investigations, and the acquisition of drug samples.

The work carried out ideally required repetition to a larger degree than was actually conducted for complete validity. In actuality, each experiment was conducted once, although in some cases repeats were run concurrently, for instance, during the identification of the organisms present within the drug samples which was felt to be of particular importance. Identification was conducted by colony examination, haemolysis patterns on blood plates, microscopic examination, including Gram staining, and catalase testing.

Particulate Content of injections

The method selected for this analysis was that of the then current British Pharmacopoeia (2000) at the start of the PhD project. This method was the method of choice for the detection and characterisation of particles present within injectable pharmaceutical preparations. The use of the BP method has provided results which are directly comparable with those for a pharmaceutical product.

The work produced clear results, with the only limitation being the lack of drug material available.

Additional Laboratory Investigations

pH

pH measurements of the injection solutions were conducted using typical laboratory grade pH measurement apparatus to measure the pH of a prepared injection solution identical to that which would be injected.

The pH of injections may vary from that of blood (venous blood pH 7.36), but the effect they have on the vessel/tissue into which they are injected would vary on the buffering capacity of the vessel/tissue. The effect the injection has upon the injection site is dependent on the injection site, and the blood has the capacity to adjust the H^+ ion concentration of an injection solution to a large degree, whereas the injection into muscle and subcutaneous tissue has a lower ability as they do not have haemoglobin present to augment the bicarbonate buffering system.

Although the pH effects of injection solutions depend highly on the type and site of the injection, this work only aimed to record the measurements found in typical injections. These relatively simple measurements, which are performed routinely on pharmaceutical injections, had not been conducted on illicit drug injections to date. The work recorded the ranges of values that can be expected to be found in typical injection solutions. The actual outcome of injection of these solutions is a more important, but unclear, factor which remains an important area for future work.

Osmolality

This was measured using the standard technique and apparatus used to confirm pharmaceutical products compliance with BP standards. The use of this method would make the measurements of the resulting injections comparable with those for a pharmaceutical product.

ES-MS

The use of this technique was conducted to investigate the constituents of the illicit heroin material. Use of this technique for this investigation was chosen through knowledge of the methodology. The paper of the work by Guilhaus et al. was found subsequently, and demonstrated that it had been employed previously for quantitative analysis of laboratory prepared opiate mixtures.

The method enabled relatively easy identification of the compounds present within the illicit samples. While it appears to be a suitable method for this analysis, validation would have to be conducted using another method to verify the contents of the samples. This was not possible during this project. Additionally, it remains to be seen how robust the method is with regard to the nature of the samples analysed. During the work, control samples of phenobarbitone and lactose were submitted for analysis, alongside the other standards. Both of these compounds produced highly complicated spectra with many peaks. From the output spectra of the illicit samples, it was clear neither of these compounds were present as there were limited numbers of peaks at m/z ratios, and they could be identified. Had either, or both, of these compounds been present within the samples, interpretation of the spectra would be highly complicated, potentially impossible, making the technique of little value. As it stands these results, along with those from Selby (1998) demonstrate the technique is of value, but if compounds that complicate the interpretation of the spectra are present in illicit samples (as they have been in the past (Kaa 1991;King 1997;O'Neil & Pitts 1992)) a different method of analysis would be required.

6.2 Project difficulties

6.2.1 Limited drug supply

The main component of all the laboratory investigations conducted during this work was the illicit drug material. It was critical to all the experiments. Previous work by Scott (2000) in Aberdeen using similar drug materials was conducted using a plentiful supply of seized illicit drug material obtained from the police with relatively little difficulty.

In the time since this work was conducted, the rules governing all aspects of controlled drugs have come under scrutiny during the inquiries into the activities Dr. Harold Shipman.

This affected the nature of this work to a great extent. Firstly, a Home Office licence had to be obtained for the storage and the work using the illicit drug material. Once this was obtained the police could be approached for supplies.

The police were very wary of supplying quantities of illicit drug to the research team, even though both members are registered pharmacists, with direct access to pharmaceutical grade controlled drugs. Before making the initial supply, criminal background checks were conducted.

Once these checks were finalised, illicit drug samples were supplied. Due to destruction requirements placed on the police in the light of the Shipman Inquiries, the quantity of heroin they had available for supply was very limited (Approximately 2.5grams). As each experiment involving the preparation of an injection of heroin would use 130mg, it is clear this limited the amount of investigations that could be conducted considerably. Additionally, some of this quantity was required for investigations into filters and preparations using different types of water, which were not part of this research project.

6.3 Limitations of findings

The main limitation of this project is the lack of depth in the majority of laboratory investigations. Given larger quantities of illicit drug material, access to

particular equipment, and aspects of specialist expertise, for instance in microbiology, the majority of work could be expanded. This leaves a large potential for future work to be conducted in this area. As has been outlined, the risks posed by the injection of illicit drugs are real and they are not going to diminish with time.

The injection preparation procedure used within the laboratory used a generalised method formed from the amalgamation of the interview participants. It does not take into account extreme procedures, for instance those using very large quantities of acid. Additionally the procedure used was formed using the preparation techniques of injectors from the South West of England. Different techniques maybe found in more distant regions, for instance Scotland. On a wider scale, the preparation techniques will vary regionally, even where acidifiers are required, such as different areas of Western Europe.

The results of the investigations using illicit drug samples are limited by the nature of the drug samples used. The use of different illicit samples with different compositions would potentially produce different results. Given the nature of illicit drugs, this limitation is impossible to overcome, as the composition changes from batch to batch.

The final limitation of the results depends on changes over time. Changes are likely to occur both in the preparation techniques of the injectors, as well as the composition of the drug material. It is possible that a change in one will change the other, for instance if heroin became available as the hydrochloride salt, injectors may abandon the use of acidifiers during the preparation process. Such a change would make the majority of this work irrelevant and require new investigations to be conducted.

6.4 Overall conclusions of the project work: revisiting the original research questions

6.4.1 How are injections produced?

There are a clear set of steps for the production of injections of each drug.

Heat is always used to prepare heroin containing injections.

The quantities of drug, acid and water used to prepare injections appear to be unrelated and vary from injector to injector.

6.4.2 What acids do injectors use?

An acid is always used to prepare the injections of heroin and crack cocaine

Citric and ascorbic acid are the most popularly used acids.

This study has recorded the spectrum of acids in use by injectors, the first time by a study.

6.4.3 What filters do injectors use?

All injections are filtered.

If Swan type filters are used, a thin strip is ripped and used, rather than the whole filter.

The standardised injection preparation method was derived based on the methods observed during the interviews. Although based on the 'average' injector's procedure, it represents a model for investigating the properties of illicit drug injections.

6.4.4 What is the drug content of prepared injections?

A method using quantitative NMR has been proposed for the measurement of the content of all components within an illicit heroin injection, although it needs further refinement.

6.4.5 What are the microbiological risks posed by illicit injections?

B. cereus and *S. aureus* were not inactivated during the preparation process using water and acid.

Organisms were found in injections prepared with drug material and citric acid. *S. aureus* appears to survive the injection preparation process using drug, the *B. cereus* result was less clear, although it appeared a related organism (possibly from the drug material) was present within the prepared injection.

Microbiological organisms are able to survive the injection preparation process. This concurs with previous work that has shown that organisms, including fungi and *Clostridia* have survived the injection preparation and been injected leading to infections.

6.4.6 Particulate matter within injections

Preparation of WFI in a manner similar to that of illicit injectors does not add significant particulate matter to an injection solution, indicating the method of preparation of injections does not pose much risk of particulate contamination, but this will be dependent on the environment.

The filters used by drug injectors add particulate matter to the injection solution, although the solutions produced remain within the limits specified by the BP, in most circumstances.

Preparation of injection with heroin resulted in large quantities of particulate matter within the solutions. The use of cigarette material did little to filter the injection suitably, but the use of Sterifilters lowered the particulates within the injections to almost with BP standards.

6.4.7 Summary

There are a number of areas in which the preparation of injections can be improved.

The use of sterile acids to reduce the possibility of the introduction of micro-organisms into injections through the use of contaminated acidifiers.

The use of lemon juice was commonly reported during interviews as having been used by injectors and it was reported as a reserve option when the acid of choice was unavailable. The use of this is actively discouraged, but it still appears to be prevalent, therefore education of injectors engaging with services is still necessary.

The use of better filters needs to be encouraged. Ideal filters are of the Sterifilter® type, or as a second line choice, clean Swan type filters ripped carefully before use.

Overall, it appears that the laboratory investigations conducted so far support current advice currently given to IDUs. The use of citric and ascorbic acids, although recommended on the ground of safety, was not supported by any scientific evidence. The work of this project has found no evidence to suggest that these acids could be responsible for problems from the given results.

6.5 Findings with respect to drug injectors

The characterisation of the preparation on procedure conducted during the first stage of this project is important for drug users for two main reasons. Firstly, this information can be disseminated to those working with drug injectors to aid care for injectors. Knowledge of, and understanding of the preparation method is important. A drug worker who is unaware of the way that injections are usually prepared will not be able to identify errors that may have arisen through problems in the method used to prepare injections.

The ripping of filters before use presents the risk of introducing micro-organisms from the injector's hands directly into the solution immediately prior to

injection. This presents the opportunity for intervention by workers who should ideally suggest that the injectors should wash their hands prior to handling the filter (ideally before starting the preparation) and also to encourage the use of only new, clean filters.

Secondly, the laboratory work conducted during this project was novel and it has investigated some of the pharmaceutical aspects of illicit injections. Through laboratory investigation, risks presented by illicit injections can be identified and suggestions introduced to prevent complications that might result.

The crucial starting point for work to improve injection safety would be study the effect of the injections upon the injectors. This would possibly take the form of *in vitro* investigations of the effect of the injection solution upon venous tissue. The effect of doses of citric and ascorbic acid could be investigated *in vivo*, comparing the effect of doses on drug injectors and healthy subjects.

As a health care professional regularly engaging with drug injecting clients, it is felt that the project outcomes are less than ideal, but it is possible that this was expecting too much. The project has taken the first tentative steps into the investigation of the pharmaceutics of illicit drug injections. It is hoped that further work will be conducted to realise the benefits that such investigations could produce, reducing harm to drug injectors, reducing risks and costs to society and reducing NHS expenditure on treating injectors with possibly preventable complications.

Once recommendations are in place to guide safer injection preparation, it will be important to educate users about the injection preparation process, to reduce their risks. This task will rest with drug workers, and through the use of leaflets available at needle exchanges and other drug services.

6.6 Findings with respect to pharmacy

Community pharmacists have unique contact with drug injectors. They may be involved in needle exchange, in which case they are intimately involved with the local injecting population. Pharmacists do not have to provide a needle exchange service to interact with drug injectors, some pharmacies sell syringes to injectors, others will sell citric acid to injectors requesting it. It is possible that these injectors are not in contact with local drug services. In addition to this, drug users on maintenance prescriptions may still continue to inject.

Pharmacists selling citric acid to persons also requesting needles and syringes are in an ideal position to provide advice on the use of acids. The outcomes of this work are of great importance to pharmacists involved in the care of the injectors they come into contact with. Dissemination of the results of this work could educate pharmacists to enable them to improve the care they provide. Now that all registered pharmacists are required to undertake compulsory continuing professional development, this work could be incorporated into training materials on drug misuse.

It would be of use to pharmacists to be aware of the methods that injectors use to prepare drugs for injection, which could possibly allow them to make interventions if they were aware of problems in the injector's preparation method. As suppliers of the acids to injectors, it is important that pharmacists understand the reason for their use. Recent unpublished research reveals that 55% (277 out of 503 surveyed) of community pharmacists do not understand the reason for the use of acid (Personal communication, Rachel Britton, 2005). Pharmacists should know which are the safer acids to use, and those which to discourage the use of. This could also potentially include advice on the use of filters too. Most importantly of all, pharmacists should monitor the injectors health as best as is possible, and they should understand common injecting problems (Scott & Bruce 1998) and encourage the injector to seek medical advice regarding complications.

In addition to educating qualified pharmacists about the work, and given the likelihood that they will be providing care for illicit drug injectors, it would be important to incorporate this work into the undergraduate pharmacy MPharm degree course. The work would teach them about the methods that drug users employ to prepare injections, the chemistry behind the process, and the role of the pharmacist in the care of drug injectors.

This project has successfully tied together the work of pharmacy practice and laboratory based science. It has demonstrated the contribution that can be made by researchers skilled in both sociological research methods, including interviewing, and laboratory investigation methods including separation techniques such as capillary electrophoresis and HPLC, and microbiology.

6.7 International considerations of the work

The preparation methods of heroin injectors investigated within this work are only applicable to areas where users prepare and inject the base form of heroin. This means the work is of little value in North America and Australasia. This however does not belittle the value of this work in these regions. The interview and laboratory investigation techniques used during this work could be applied to investigate local preparation techniques and injection samples prepared using the local methods. This would provide insight into the risks from those injections and allowing an international comparison of the risks posed to illicit heroin injectors.

Crack and freebase cocaine exist as the same form in all countries, therefore work conducted on this would be directly comparable. The variations in methods used to prepare crack in different countries would be the important sections of this work were it to be conducted.

6.8 Further Work

The laboratory investigations conducted during this project should serve as a starting point for future work into illicit drug injections.

The microbiology investigated would benefit from further work within a dedicated microbiological department or facility. This would provide equipment and staff with appropriate specialised training. This would result in the ability to conduct work on a greater scale and allow accurate identification of a greater range of organisms.

The particulate matter investigations suggest that a portion of the particles within the samples were contributed by the filters used during the preparation process. Identification of the matter within the injection solutions would provide a clearer understanding of this. Such investigations could potentially be conducted through the use of Raman mapping techniques.

Identification could enable the identification of the particulate content and allow tracing its source.

The effect of the injection solutions upon the tissues into which they are injected is a logical progression on from the study of the injection solutions themselves. It is important to understand the effects they produce on them and which properties of the injections are responsible for these effects. Such work will further guide injection preparation advice provision to IDUs.

These effects could be investigated in a number of ways. Models for investigating the effects of infusion induced phlebitis have been developed that use the effect of the injection solution upon buffer solutions, without the use of animal tissue. Alternatively, the use of cultured cell lines, or venous tissues from animals could be used and histological examination used to assess the effects of the drug solutions.

The use of citric and ascorbic acids by IDUs to prepare injection solutions have unknown biological and pharmacokinetic effects. It is postulated that the acids pose no risk and are metabolised by the body without problem, but this is unproven. If this metabolism does not occur there would exist the risk of ascorbate and citrate levels increasing within the body. *In vivo* studies using health volunteers could be used to investigate the consequences of regular dosing with these substances to confirm that their use by IDUs poses no risks in this respect.

The interviews conducted investigated the preparation of crack and 'speedball' injections, along with that of heroin. Crack was unavailable for use during the laboratory stage of the project, so injections were not investigated. The interviews were lower in number than those for the heroin which indicates further interviews into the preparation of crack containing injections may need to be conducted. This will then enable laboratory work to investigate the properties of the injection solutions. Investigations of the same nature, and the same technique, where applicable, would provide information on the risks posed by these injections.

The microbiology of crack is especially of interest, given that it is commonly carried orally or rectally by drug dealers. This will lead to the possibility of the drug or its wrapping material being contaminated with bacteria

from the oral or rectal cavity. This may result in the organisms transferring to an injection solution.

Finally, future work educated IDUs on the safest method of injection preparation would be the ultimate goal of work within this area. This would see the transfer of the results of this project as a whole being transferred into the safer practice it aimed to introduce.

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Appendix 1

Ethics approval letters and Hereford PCT approval letter

Royal United Hospital Bath NHS
NHS Trust

BATH LOCAL RESEARCH ETHICS COMMITTEE
Direct tel/fax: 01225 825725 email: research.ethics@ruh-bath.swest.nhs.uk

Royal United Hospital
Combe Park
Bath
BA1 3NG

Tel: 01225 428331

19 April 2002

Mr R Ponton
Research Pharmacist
Department of Pharmacy & Pharmacology
University of Bath
Bath BA2 7AY

Dear Mr Ponton

BA242 (*(please quote this reference on all correspondence)*
Illicit drug injection preparation procedure investigation using a questionnaire and practical demonstration

Thank you for your letter dated 16 April 2002 enclosing a revised volunteer information sheet. It was the Committee's view that it would not be necessary for you to obtain written consent from the volunteers because of the anonymised nature of the study. The Committee also did not think that the option whereby the volunteer marks or initials the consent form would have any legal basis. I am pleased to confirm that you have fulfilled the requirements of the Committee and your study has full approval to proceed. I would be grateful if you could reference the revised information sheet 'Bath version April 2002'.

This Committee is organised and operates according to ICH/GCP Guidelines and the applicable laws and regulations. Any changes or extensions to the protocol, or additional investigators should be notified to the Committee for approval. Serious and unexpected adverse events should also be notified to the meeting. May we remind you of the Data Protection Act 1998 and the need to conduct the trial in accordance with the Good Clinical Practice Guidelines.

The Committee is required to audit progress of research and to produce a yearly report to the Health Authority and Department of Health. You are therefore required to provide a brief yearly report and a short final report.

Yours sincerely

Anna Jenkins

pp **Dr Andrew Taylor**
Vice-Chairman

PCT004

Herefordshire NHS
Primary Care Trust

Victoria House,
Eign Street,
Hereford,
HR4 0AN.

Tel: 01432 262008 (DD)
Fax: 01432 341958

AS/JPD/C7b(i)

1st July, 2002.

Mr. R. Ponton,
Research Pharmacist,
Department of Pharmacy & Pharmacology,
University of Bath,
Bath,
BA2 7AY.

Dear Mr. Ponton,

EVIDENCE-BASED HARM REDUCTION (STAGE ONE):

I am pleased to confirm that the Herefordshire District Ethics Committee considered your application in respect of the above study at its meeting on 20th June, 2002.

Following discussion, the Committee gave its approval to your study and a report on its outcome would be welcomed in due course.

The Committee did request that your attention should be drawn to its concern at some of the wording on the first page of the "Information for Patients" Sheet in that the wording of the requirements for "Part Two" were not clear.

Please accept my apologies for not responding sooner – I hope that your face has returned to normal now following your operation!!

Yours sincerely,

Janet P. Dickinson

Mrs. J. P. Dickinson,
Administrative Services Manager.

Herefordshire NHS
Primary Care Trust

Herefordshire Mental Health Services
Stonebow Unit
County Hospital
Hereford
HR1 2ER

Tel: (01432) 364127
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CJT/MAW

23rd July, 2002

Mr. Rhys Ponton,
Research Pharmacist,
University of Bath,
Dept. of Pharmacy and Pharmacology,
Bath, BA2 7AY

Dear Mr. Ponton,

Thank you very much for completing the form on Research Governance. I apologise if this seemed a rather bureaucratic procedure but I am afraid it is now Government requirement that all research projects undergo particular scrutiny to ensure that standards and other aspects of governance are being maintained.

I have read through the information you have provided and confirm that as far as I can tell your research does comply with all of the Research Governance standards that are expected. Your research also seems to be a very interesting and exciting project and I very much wish you success with it. I note that you are working closely with Danny Morris, but if I can be of any assistance in helping you with your research whilst you are carrying it out in Herefordshire do not hesitate to contact me.

I have to inform you that as part of the Research Governance procedure it is possible that during your research either I or one of my colleagues may contact you to check that the standards are being maintained. This is routine procedure and could apply to any research carried out within the Herefordshire region.

Finally, because your research does seem so interesting and of great value to mental health, I would be very grateful if I could have a copy of any publication that results from this research. It might also be very interesting if you would be prepared to give a presentation on the results of your research at some stage in the future to the Herefordshire psychiatric department.

With best wishes,

Yours sincerely,

Dr. Chris Thomas
Consultant Psychiatrist

UBHT Headquarters
Marlborough Street
Bristol BS1 3NU
Tel: 0117 928 3613
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Email: naaz.nathoo@ubht.swest.nhs.uk

United Bristol Healthcare NHS

NHS Trust

25 October 2002

Mr R Ponton
Research Pharmacist
Department of Pharmacy & Pharmacology
University of Bath
Bath
BA2 7AY

Dear Mr Ponton

E5450 Illicit drug injection preparation procedure investigation using a questionnaire and practical demonstration (RECIPROCAL)

Thank you for your email communication dated 21st October 2002. Your comments have been reviewed by a a Sub-Committee of the UBHT LREC who are now happy to grant full approval to the above study.

We wish you success with the research and look forward to knowing the outcome in due course.

In accordance with Good Clinical Practice Guidelines of the European Community and the standard operating procedures required by NHS(E), the LREC is required to monitor research. The International Conference on Harmonisation Tripartite Guideline requires an annual, as well as end-of-study report. Please complete the enclosed project report at the end of the study or after each year from the beginning of the study and return it to us. Continued approval depends on the receipt of these reports.

This Committee is compliant with ICH/GCP Guidelines except when illness or lack of resources prevent this. Any changes or extensions to the protocol, or investigators should be notified to the Committee for approval. Serious and unexpected adverse events should also be notified.

Investigators who undertake research within the Trust and subsequently leave the Trust are reminded that they must not take with them patient information unless it is anonymised such that individual patients cannot be identified without reference to the Trust.

Reminder: The title will be published in national and Trust registers. It should not contain confidential information that you or any sponsors of this research would not wish published.

Yours sincerely

David Grier

D Grier
Chairman to the Research Ethics Committee

Appendix 2

Questionnaire

UNIVERSITY OF BATH

Evidence-based harm reduction

SECTION I

INJECTION PROCEDURE INVESTIGATION QUESTIONNAIRE

SITE CODE ☐

INTERVIEW NO. ☐

DEMOGRAPHIC DETAILS

1. How old are you? ____ Sex: M ☐ F ☐

DRUG HISTORY

2. What is your main drug used by injection?

Heroin	
Crack	
Heroin + Crack (Speedball)	
Other:	

3. How long have you been using your main drug (in total)? ..... years .....mths

4. How much do you use per day? £ ____ No. of bags/rocks ____

5. Do you use any other drugs as well at the moment?

	Crack /Cocaine Freebase (if NOT drug of choice)	Powder Cocaine	Heroin (if NOT drug of choice)	Amphetamine	Other .....
Used					
Injected					

INJECTING HISTORY

6. How long have you injected? ..... years .....mths

7. What drugs have you ever injected?

Then Prompt:

Heroin	
Cocaine (powder)	
Cocaine freebase/Crack	
Heroin + Cocaine (Speedball)	
Amphetamine	
Benzodiazepines e.g. diazepam, temazepam, (vallies, temazzies, jellies)	
Cyclizine	
Diconal	
~~Dihydrocodeine~~	
~~Subutex/Temgesic~~	
Tablets	
Other drug	
Other drug	

8. How many times a day do you inject your main drug?

9. Where do you inject on the body?

10. How did you learn to prepare drugs to inject?

THEN PROMPT: Were you given specific details/quantities to use, or did you learn through 'trial and error'?

11. Which drug did you first inject with?

Heroin	
Cocaine (powder)	
Cocaine freebase/Crack	
Heroin + Cocaine (Speedball)	
Amphetamine	
Benzodiazepines e.g. diazepam, temazepam, (vallies, temazzies, jellies)	
Cyclizine	
Diconal	
~~Dihydrocodeine~~	
~~Subutex/Temgesic~~	
Tablets	
Other drug	
Other drug	

12. Where did you learn to do this?

[locality and country]

INJECTION PREPARATION

13. How much drug do you usually inject for each 'hit'? £

14. Do you use an 'acid' to prepare your injection? Yes ☐ No ☐

15. Why do you add an acid? Please explain

16. Which acidifier do you prefer to use?

17. What acidifier do you normally use?

18. Where do you obtain this?

19. What do you use when you run out, or can't get hold of your usual acid?

20. What else have you ever used to dissolve the drug?

Then Prompt:

Citric acid	
Ascorbic acid	
Vitamin C powder	
Lemon juice	
Fresh squeezed lemon	
Vinegar (Brand:.....?)	
Non brewed condiment	
~~Baby Bottle Cleaner~~	
Other	
Other	
Other	

21. Have you ever had any injecting problems related to the acids?

Then Prompt:

Abscesses?	
Eye Infections?	
Burning sensations?	

Appendix 3

Demonstration questions

UNIVERSITY OF BATH

Evidence-based harm reduction

Checklist Questions

1. *What do you place in the spoon first?*
2. *How do you judge how much drug to use?*
3. *What do you use to measure this amount?*
4. *Do you do anything to the drug powder before you prepare it?*
   PROMPT: Crush it?
   If so using what?

I need to understand the decisions that you make when adding the acid-

5. *Can you explain any factors that lead you to use more or less acid?*
6. *How do you decide how much acid you need to use?*
7. *When do you know you need to add more acid?*
8. *Does amount change with amount/quality of drug?*

   If so, by how much?
   Double?
   Triple?

9. *Is acid added to drug powder in cooker?* ***Before Water****?*
10. *Which acid would you choose to use?*
11. *How do you gauge the amount of acid to use?*
12. *Does amount change with amount/quality of drug?*
13. *If so, by how much?*
    Double?
    Triple?
14. *Water added to drug in cooker?*
15. *When do you add water?*
16. *How do you measure the amount of water you use?*

17. *If Syringe is used- what size?*
    If Needle is fitted- what size?

18. *What is your normal source of water?*

19. *How much water do you use to prepare an average injection for one person?*

20. How **long** is solution usually **heated**?

21. When is the solution ready?
    When solution goes clear?
    After boiling for a certain time?

22. Is solution allowed to cool after heating at all?

23. *What do you use as your 'cooker'?*

24. *Do you heat the mixture?*

25. *Do you stir the mixture before heating?*

26. *Do you stir the mixture during/after heating?*

27. *What do you use to stir it?*

28. *What do you use for the heating?*

29. *Do you filter the injection?*

(If Applicable)

30. *What do you use to filter it?*

31. *Do you reuse filters?*

32. *How long do you store them for?*

33. *Do you change the needle before injection?*

Appendix 4

Information sheet for volunteers

UNIVERSITY OF BATH

Evidence-based harm reduction

Information for Volunteers

Safer Injecting Study

About the study

This is a harm reduction project. It aims to study the injection preparation process and prove the safest ways to prepare drug injections so injectors can be given accurate advice. You are invited to help with the work.

When you prepare injections of drugs, you follow a procedure that is still not fully understood by non-users. You may have been doing it for years and it may be second nature to you now, but to non-users the details are largely unknown. This means that any problems that might occur during or be caused by the process might not be recognised because drugs workers don't understand the technique.

To help drug workers give accurate advice, this project aims to study the process and identify any potential problems that it may cause- we can then issue advice to users on how to improve things. This interview is the first part of the project- to learn how people prepare injections, then the second part will be done in the laboratory to copy the process and test the injections made. The safest injection preparation technique tested will then be published in safer injecting leaflets.

The interview will be divided into **two** parts :

-Part **ONE** will be a short questionnaire.

-Part **TWO** will be a practical demonstration by you of your process for the researcher. This part can also be completed without the demonstration like part one, if you prefer.

Part One- The Questionnaire

This consists of some questions to establish some background information about your injecting habits. This information is concerned with some details, such as how long you have used drugs and which ones you use. As the study is centred around the problems associated with injection preparation, there are then questions on where and how you learned to prepare injections, and also what equipment you use to do it.

Part Two- The Demonstration

In this part of the interview you will be asked to demonstrate the preparation of an injection using fake drug powder.

Whilst you do this, the researcher will record the way you do things, such as how much water you use and what order you carry it out. You might also be asked questions about what is done.

The information that is collected from you and other people will then be used to recreate the preparation process in the laboratory, so that scientific tests can be performed, for example to find out which acidifier presents the least risk to health.

This understanding of user techniques and the problems that can occur will then enable us to develop safer injecting information for drug workers and guides for drug users.

Confidentiality

All information you provide, or that is noted during your demonstration will not be marked with anything that can identify you. Your name will not be asked during the interview. After the study is finished, all records will be destroyed.

At any point during the interview you can choose to leave, you do not need to give a reason. Leaving an interview or refusing to take part will not affect the treatment you receive at the needle exchange. Any information you disclose will not be given to the workers at the exchange or any other person.

If you have any questions about taking part, ask a member of agency staff who will be able to pass questions on to me, or ask me on the days I am at the agency.

Thanks very much for your time.

Rhys
PhD Student
University of Bath

Appendix 5

Demonstration checklist

UNIVERSITY OF BATH

Evidence-based harm reduction

Section II

Injection Procedure Investigation Checklist

Site Code [ ]

Interview no. [ ]

Materials

Drug: Heroin ❒
Crack ❒

Acid Citric❒
Ascorbic❒
Lemon Juice❒
Fresh Lemon❒
Vinegar❒
Other……

Water:
Tap❒
Boiled/cooled❒
Bottled ❒
Ampoule❒
Other…… ❒

Equipment

Cooker :
Spoon ❒
Drinks can❒
Bottle cap❒
Other……..

Syringe:
0.3ml ❒
0.5ml ❒
1.0ml ❒
2.5ml ❒
5.0ml ❒
10ml ❒

Needle Used? Yes❒ No❒

Needle size used:
Brown ❒
Orange ❒
Blue ❒
Green ❒
Syringe with fitted needle ❒

Heat Source:
Matches ❒
Lighter ❒
Candle ❒
Burning swabs❒
Stove ❒
Other……...❒

If **Stirred, using**:
Finger ❒
Match ❒
Syringe ❒
Needle sheath❒
Other ……❒

Filter used:
Cigarette filter❒
Roll up filter❒
Cotton wool❒
Other……❒

Procedure

Order of mixing components:
Drug/Acid/Water ❒
Drug/Water/Acid ❒
Water/Drug/Acid ❒
Other……

DRUG

How is amount of drug gauged:
- used as bought quantity ❒
£5❒ £10❒ £20❒ Other ……

- poured ❒
- using device -spatula ❒
-Other ❒ ……

Is anything done with the drug before it is used: Yes❒ No ❒

Is it **crushed** in any way? Yes❒ No❒
If so, what with?
-spoons ❒
-other ……

ACID

Added to drug powder before water? Yes❐No❐

Amount of acid used:
- poured (guessed) ❐
- pinched with fingers ❐ No. of pinches: .....
- using utensil- spatula ❐
  -Other.....

Are the powders mixed at all? Yes❐ No ❐

WATER

Drawn up in syringe? Yes ❐

Amount drawn up:.....

For use by one person only?❐ No? How Many:.....

Water added to drug in cooker? Yes❐ No❐

IF added to drug ***before acid***, is solution:
Stirred ❐
Heated ❐

COOKING

Is heat applied to solution? Yes❐ No❐

Is solution **stirred before heating**? Yes ❐ No❐

Is solution **stirred during/after heating**? Yes ❐ No❐

How **long** is solution usually **heated**? .....

When is the solution ready?
When solution goes clear?❐
After boiling for a certain time? ❐ .....seconds

Is solution allowed to cool after heating at all? Yes ❐ No ❐

FILTERING

Is **solution filtered** before use? Yes❐ No ❐

Is needle changed on the syringe before drawing up? Yes❐ No ❐

How much of material is used for filtering? .....

Is filter kept for reuse later? Yes ❐ No❐

If yes, **ASK** for how long: .....

Is needle changed on syringe before injection? Yes❐ No❐

Appendix 6

Research Protocol

UNIVERSITY OF BATH

Evidence-based harm reduction

Injection preparation process investigation interview protocol

Suitable volunteers will be recruited from the selected needle exchange agency by being made aware of the work through the 'information for volunteers' sheets. These will be available from the agency for at least one week before the interview work commences. During this time, the users will be informed by agency staff of when and where the interviews will take place. The potential interviewees will be able to ask questions that will be answered by the researcher who the agency staff will be able to contact.

The Interviews will take place on the specified dates at the agency, in a room that has specifically been set aside for that purpose during the time.

On the dates of the interviews, prospective volunteers will be asked if they have read the information sheet, and if they still wish to take part. It will then be checked that they meet the inclusion criteria- that they are over eighteen years old, that they have injected for over three months and that they prepare their own injections.

If they are suitable, exact details about the interview will be explained. They will be informed what is required of them and the aims of the work will be set out. It will be explained that the work is voluntary and that they can refuse to take part. They will also be told if they agree to participate, they can withdraw at any point without giving a reason. They will be informed that declining to take part or withdrawal will not affect the way they are treated by the service. It will be pointed out that the practical nature of the optional second part of the interview might induce craving. If they then feel this might be a problem for them, they will be given the option of just answering questions to fill in the checklist, without seeing the props. If they feel it will not be a problem using the props, they will be reminded they can pull out of the interview at any point.

Verbal consent will be obtained from them, and be recorded as having been given.

The first section of questions will then be asked.

After completion of these questions, they will then be asked if they still wish to proceed with the prop work. If they do, they will be presented with some water, fake drug and various acidifying agents. They will also have other equipment available to use such as spoons, syringes, and various filter materials. They will be asked to follow the technique they normally use to prepare an injection of the fake drug. The researcher will observe this and mark the procedure steps they follow on the checklist.

If they choose not to use the props, they will be asked about the procedure they would follow verbally.

The interview will then be concluded.

The volunteer will be thanked for their time. A selection of currently available harm reduction guides relating to safer injecting and injection preparation will be on display for the users to take with them if they desire.

Appendix 7

Mass Spectra

Sample Identification

At the top left of each spectrum there are the words: 'File Text: Ponton' followed by a letter. This letter identifies the sample under analysis. The table below presents the code letter given to each sample.

A	Lactose	(Sigma)
B	Morphine	(Macfarlan Smith)
C	Codeine	(Macfarlan Smith)
D	Street heroin pot A	
E	Street heroin pot B	
F	Street heroin pot C	
G	Phenobarbitone	(Sigma)
H	Diamorphine	(Evans/Celltech)
I	Caffeine	(Sigma)
J	Paracetamol	(Sigma)
K	Noscapine	(Sigma)
L	Papaverine	(Sigma)
M	Acetylcodeine	(synthesised in department)
N	6-Monoacetylmorphine	(Norfolk and Norwich Hospital)
O	Street heroin pot A	
P	Street heroin pot B	
Q	Street heroin pot C	

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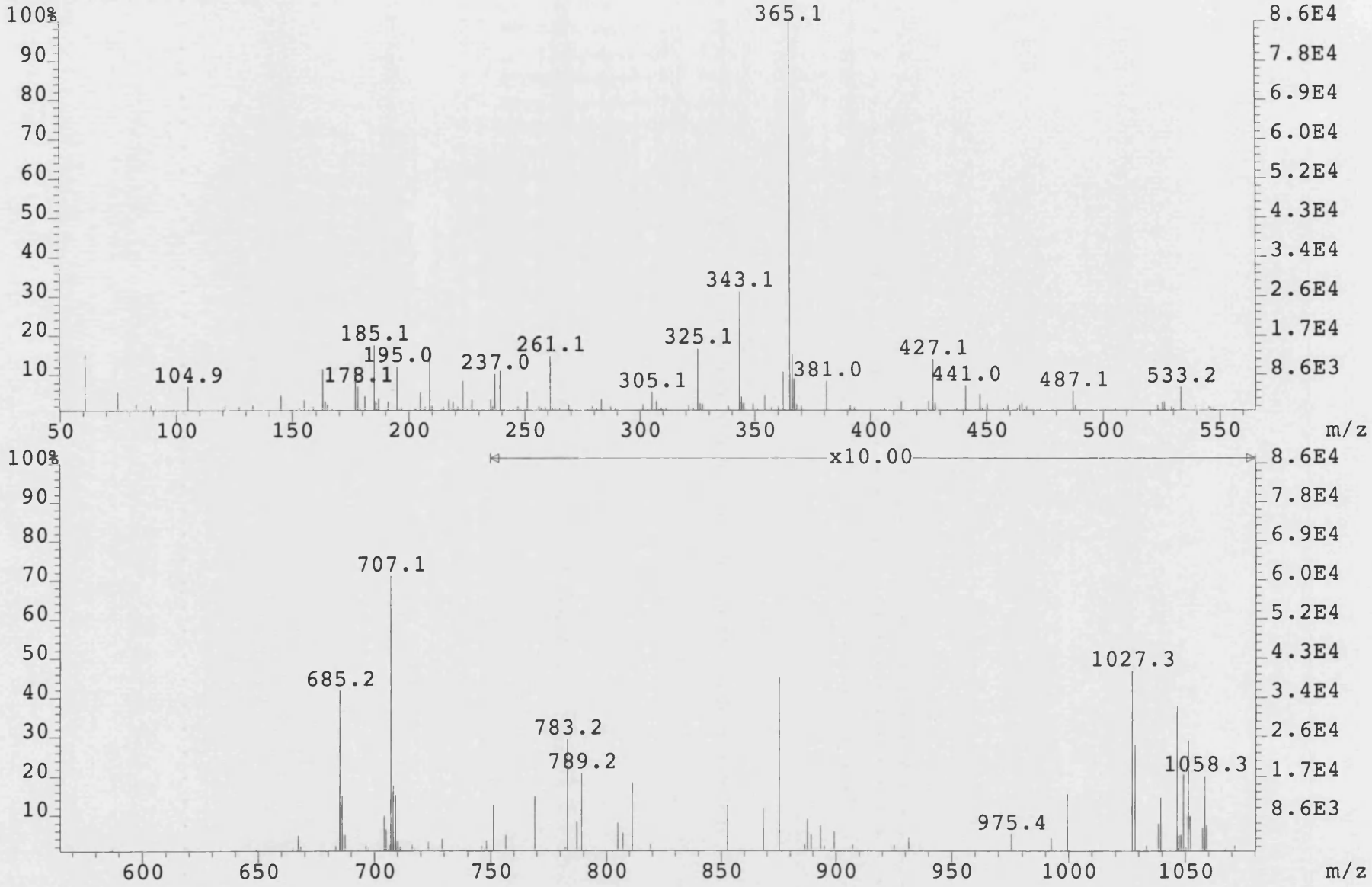

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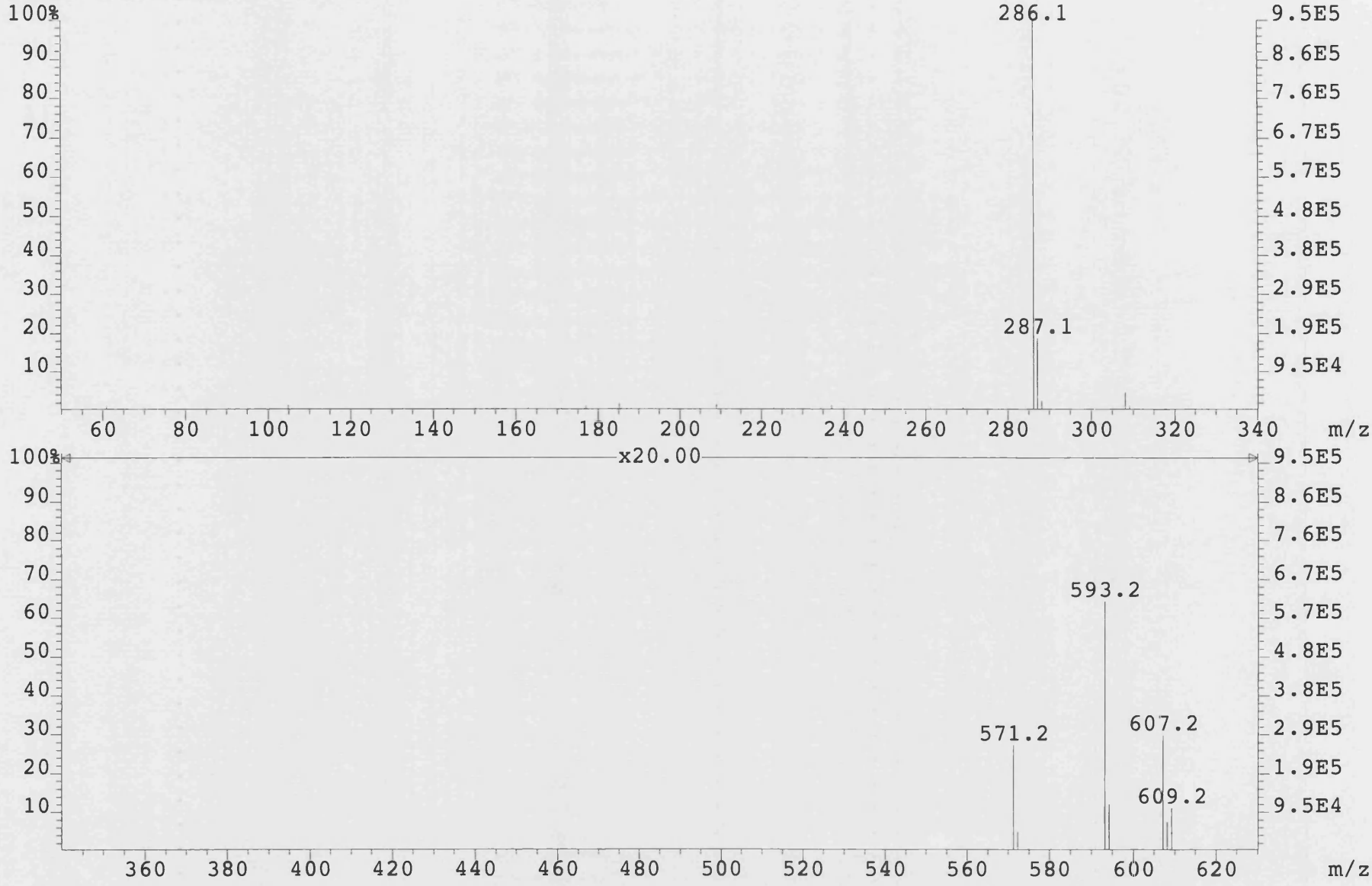

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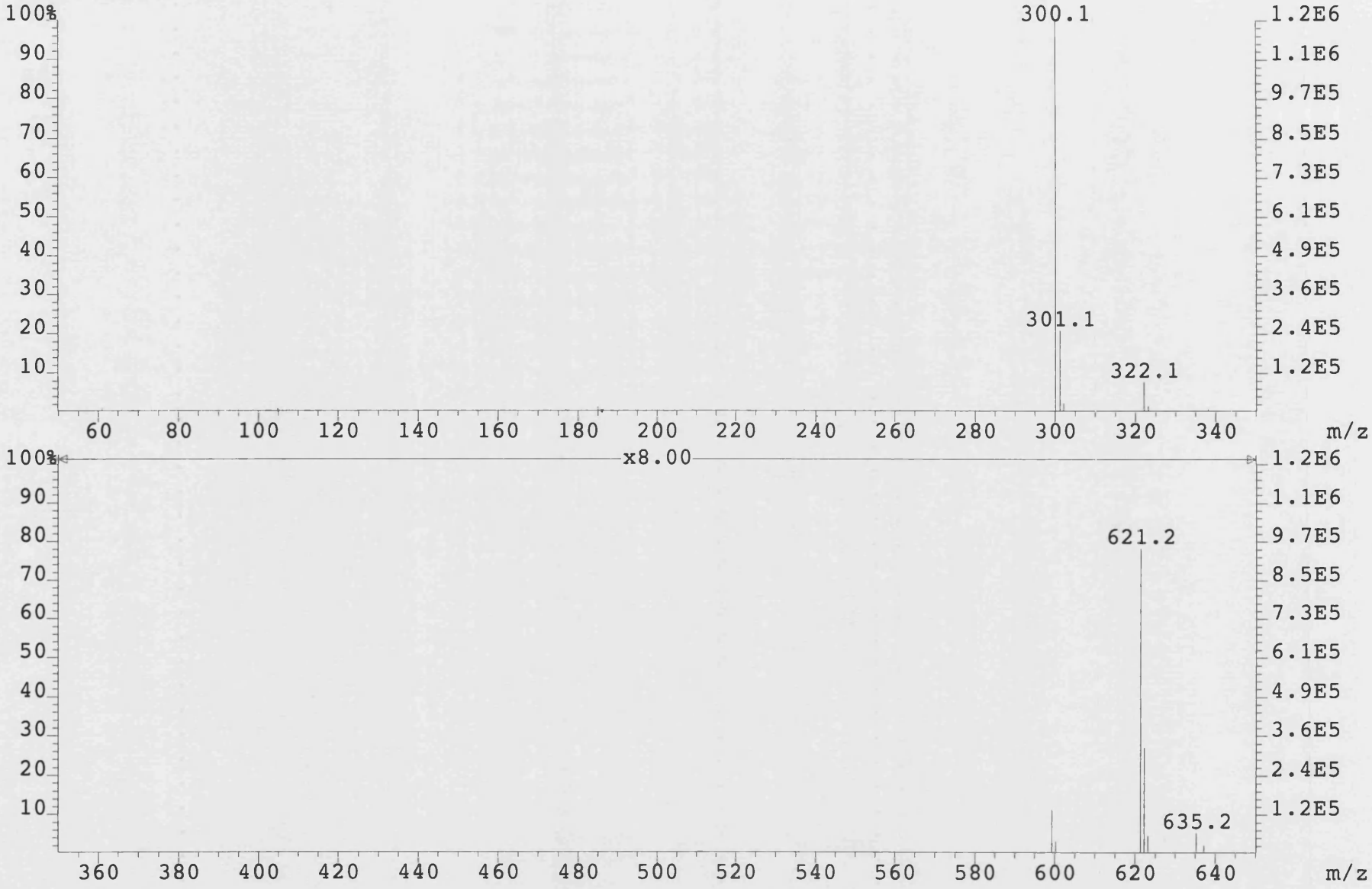

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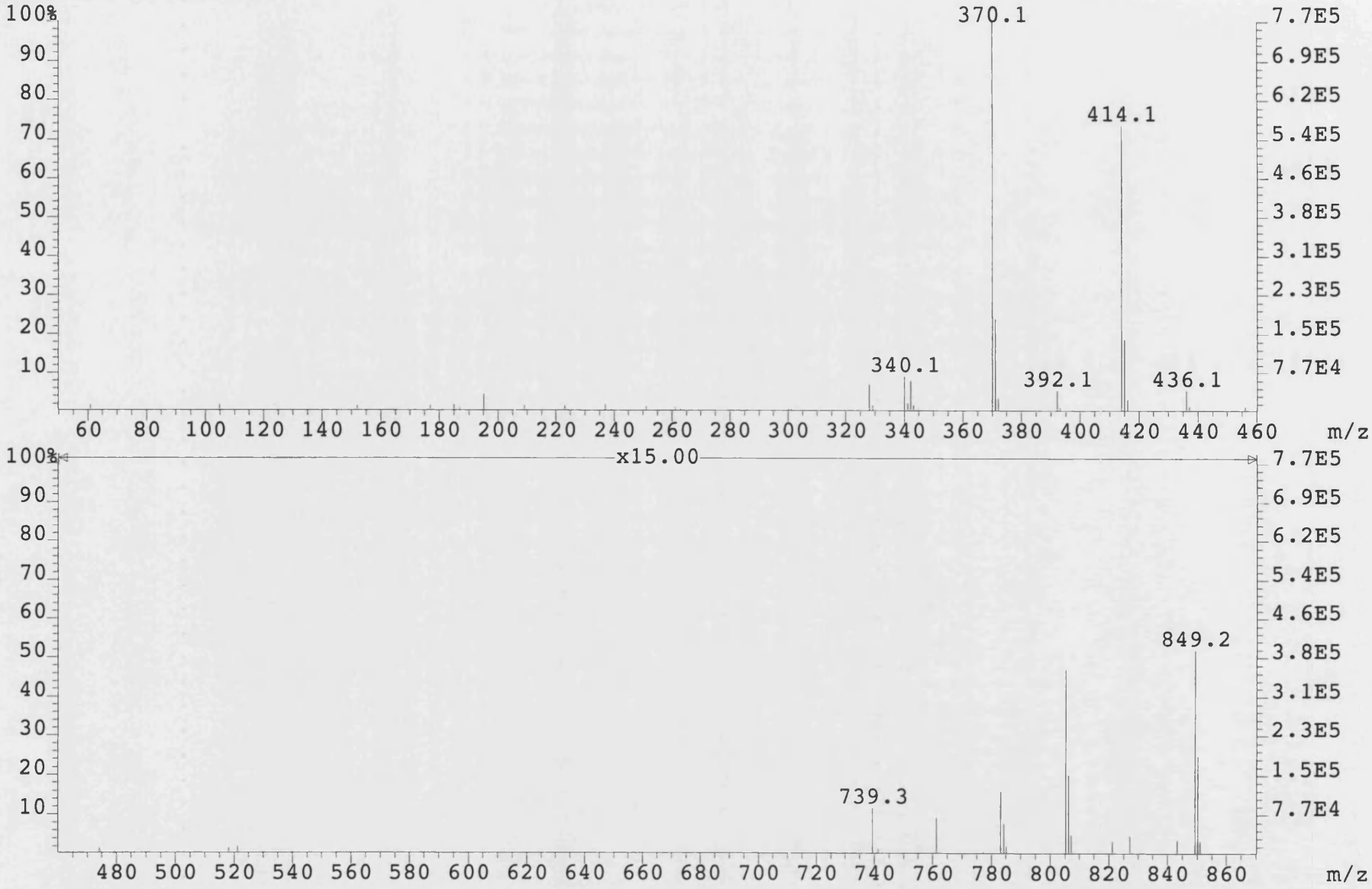

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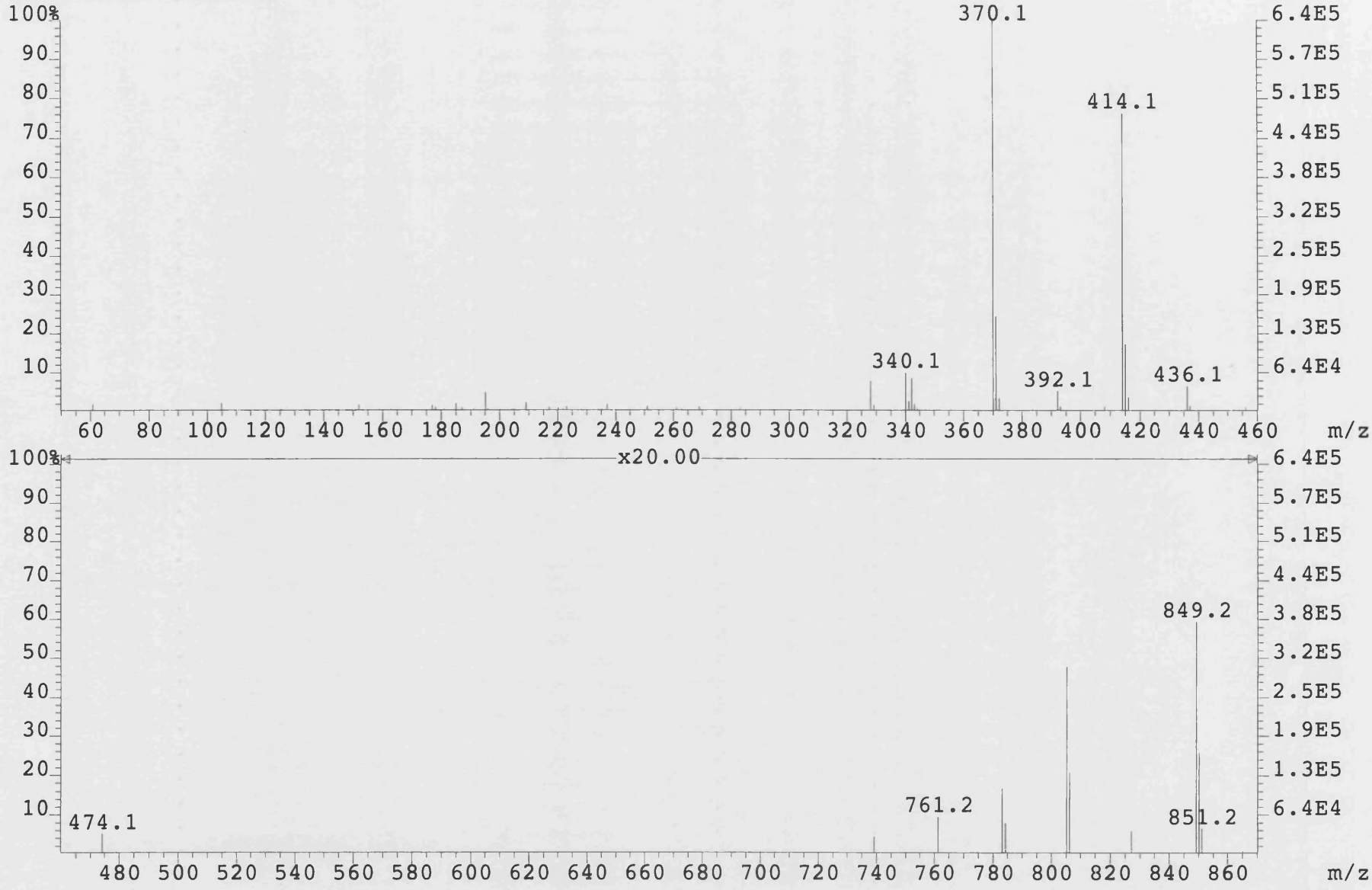

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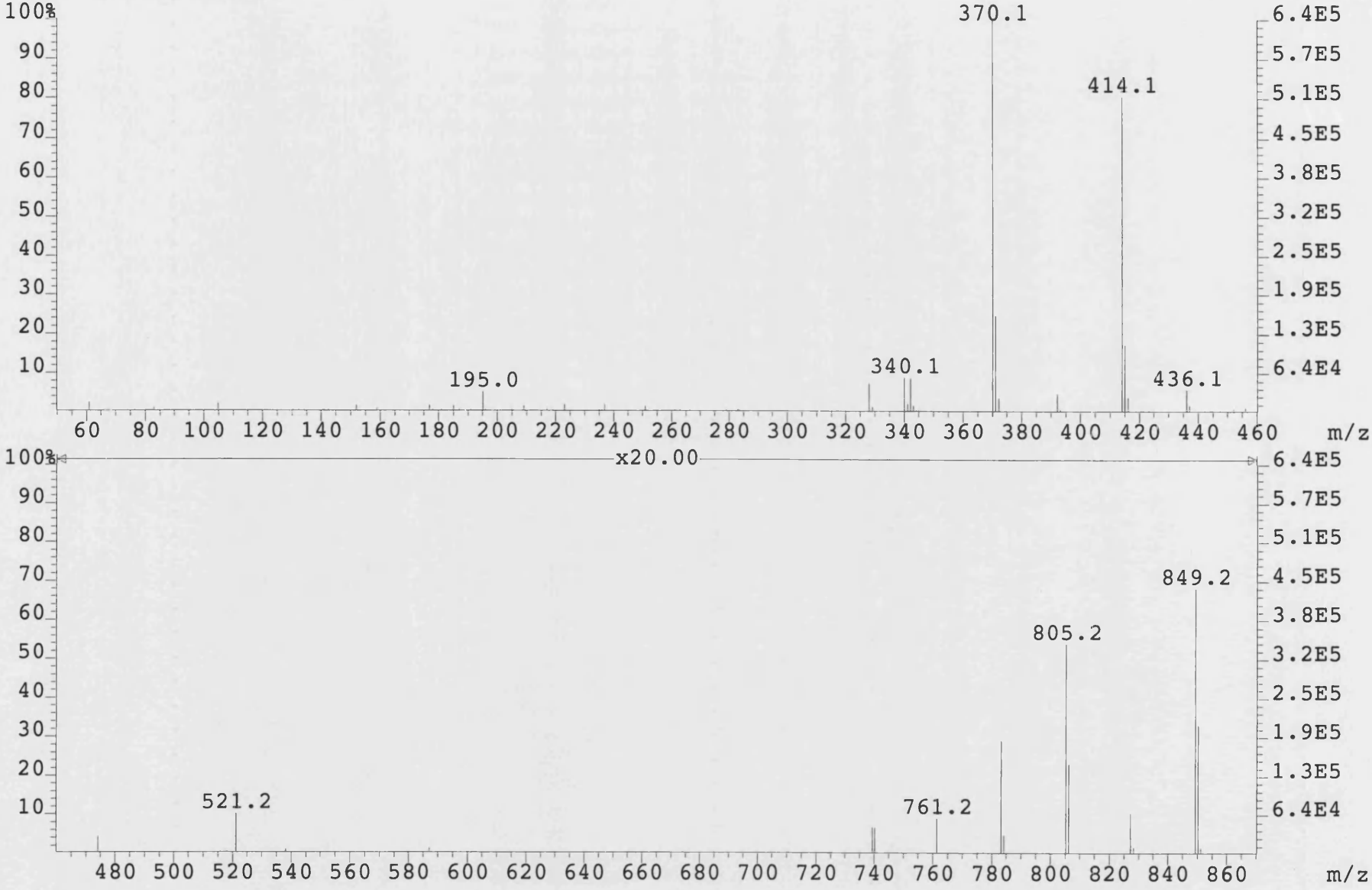

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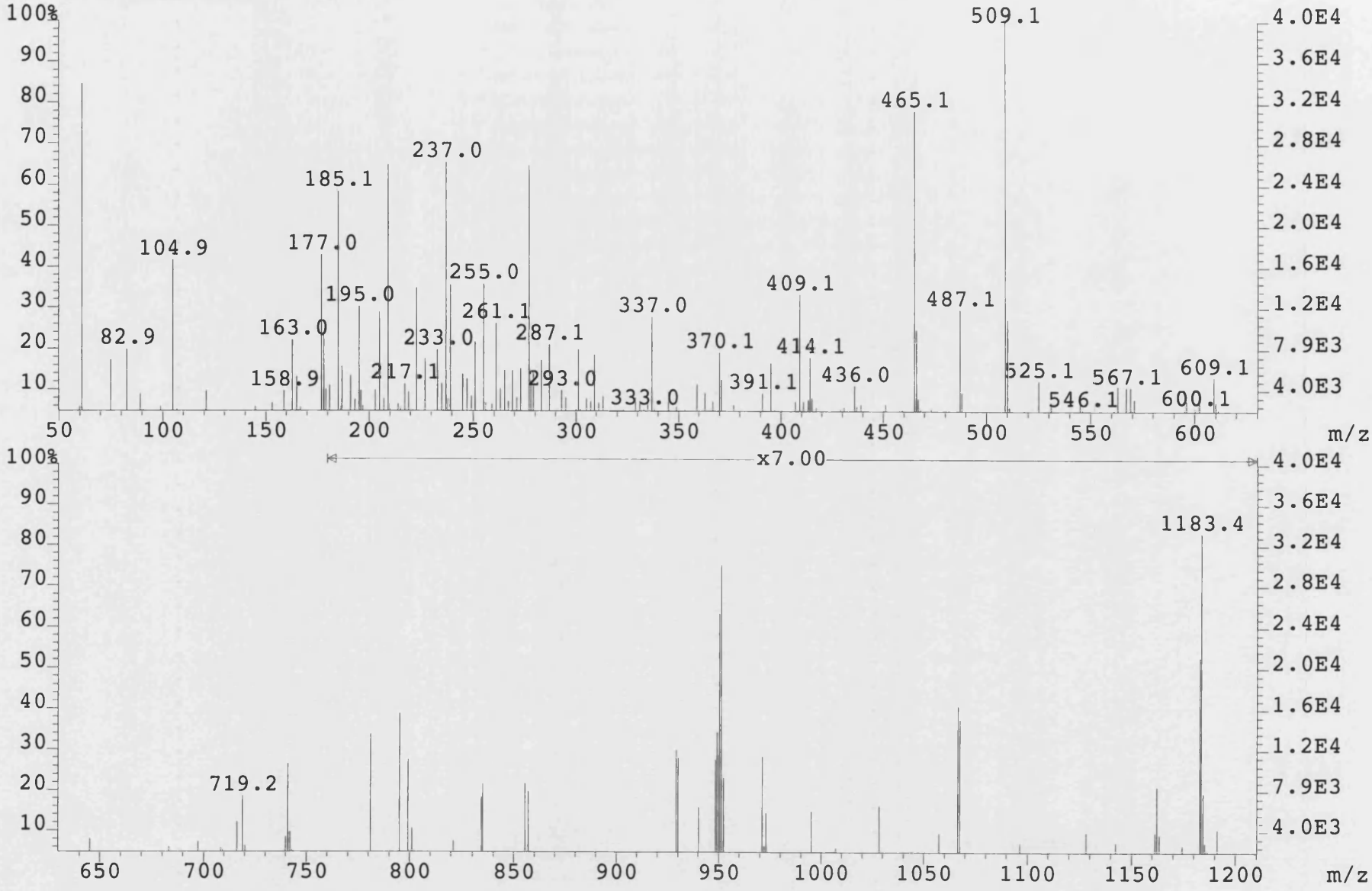

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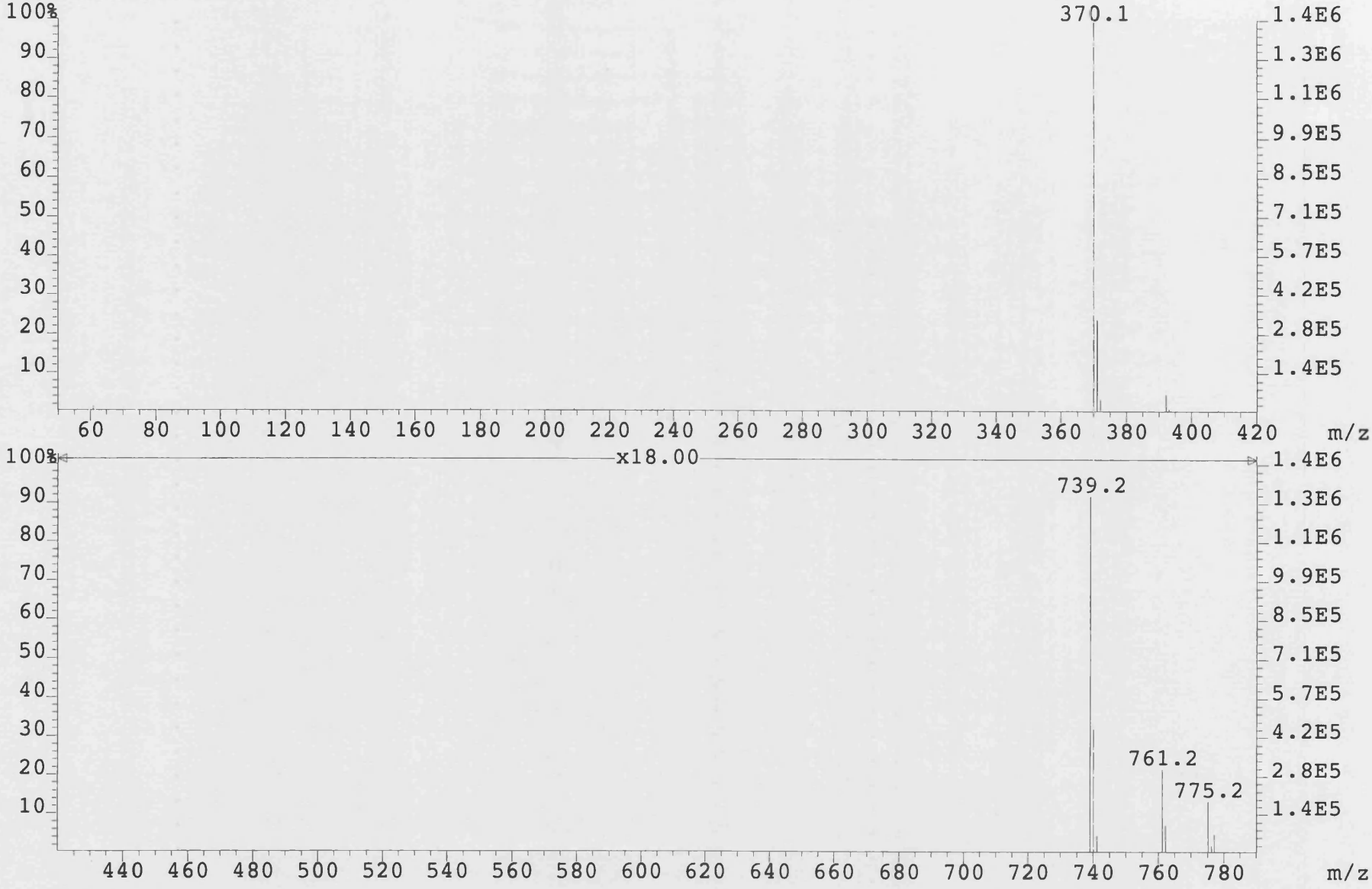

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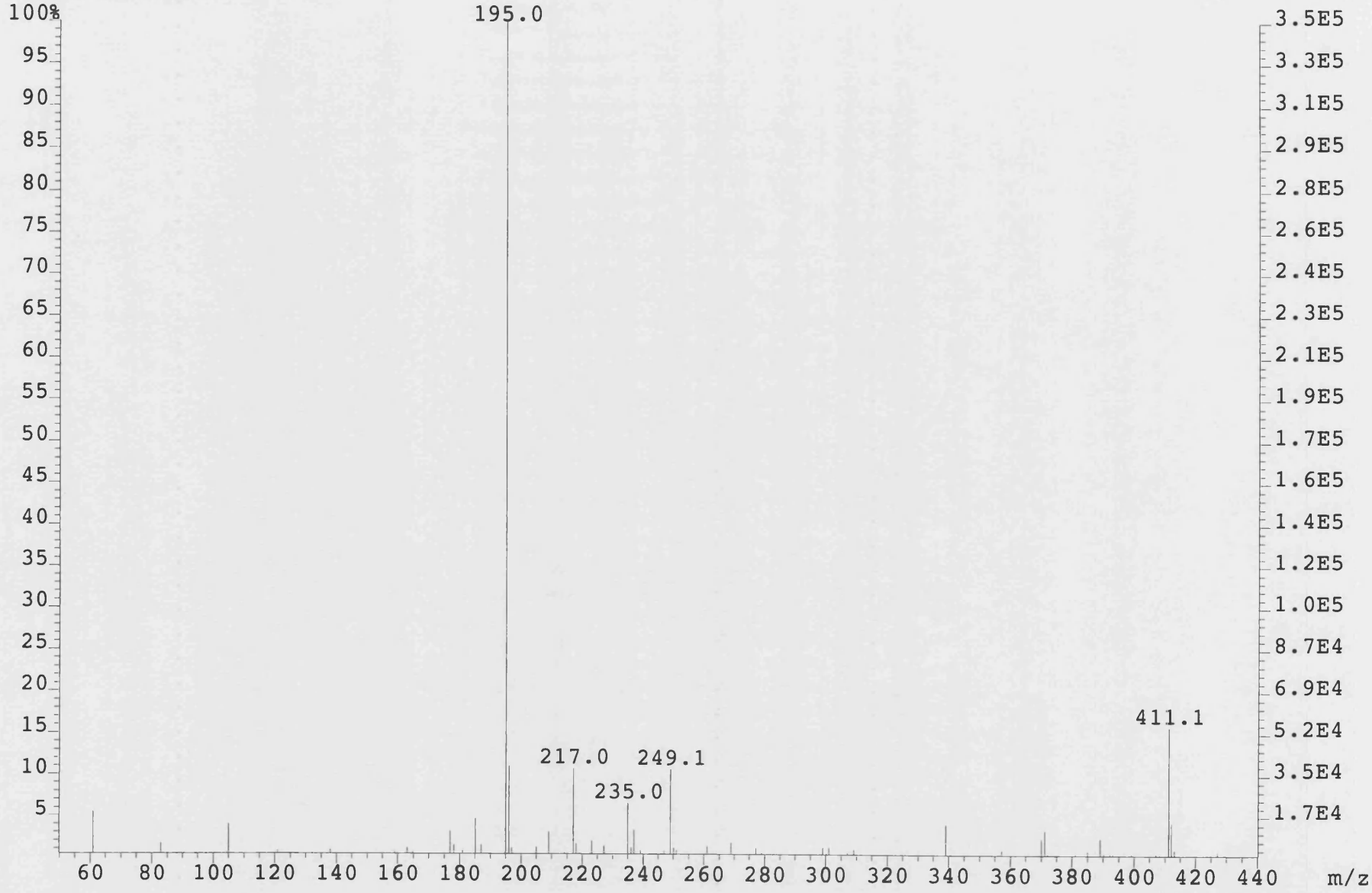

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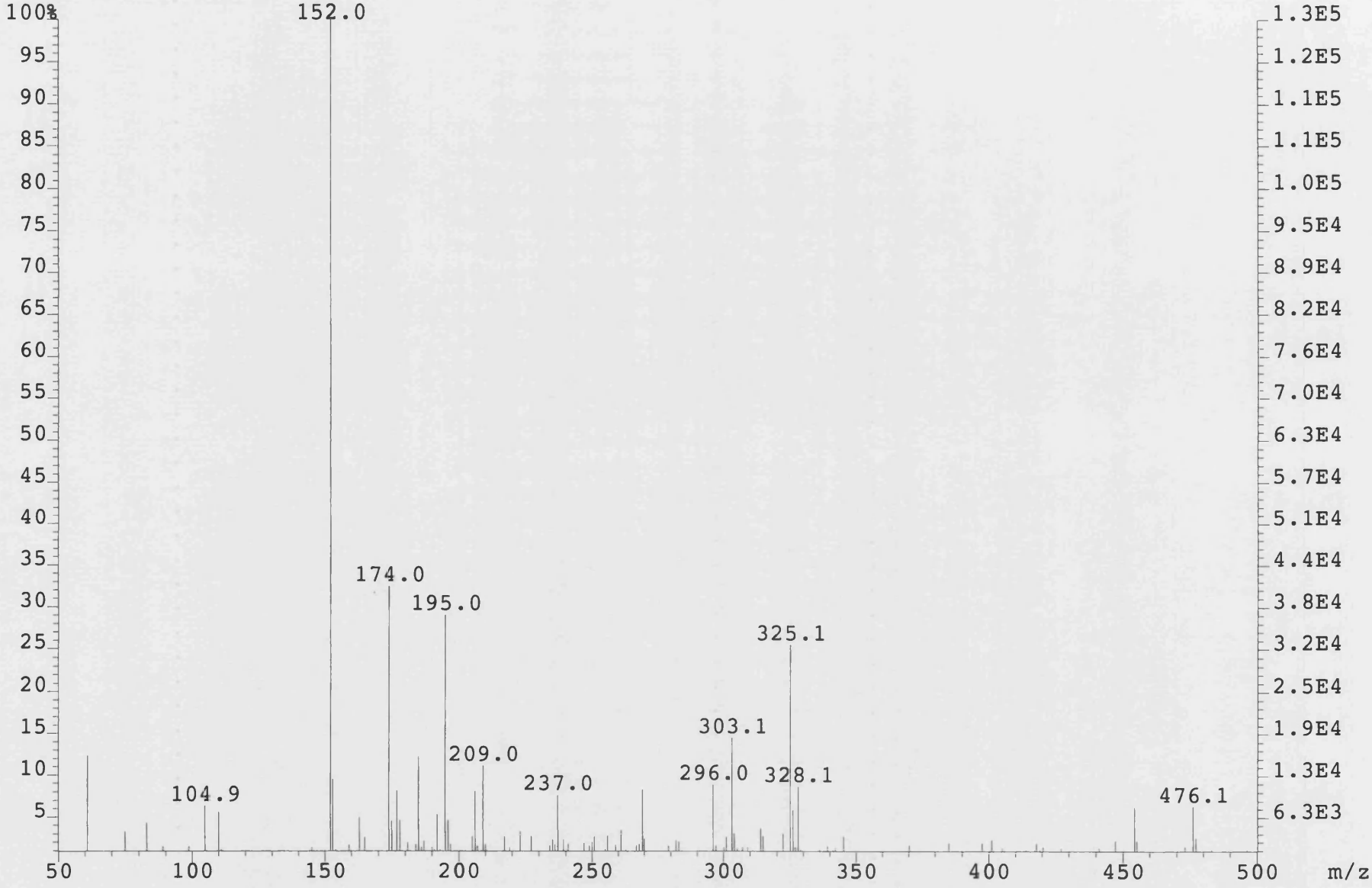

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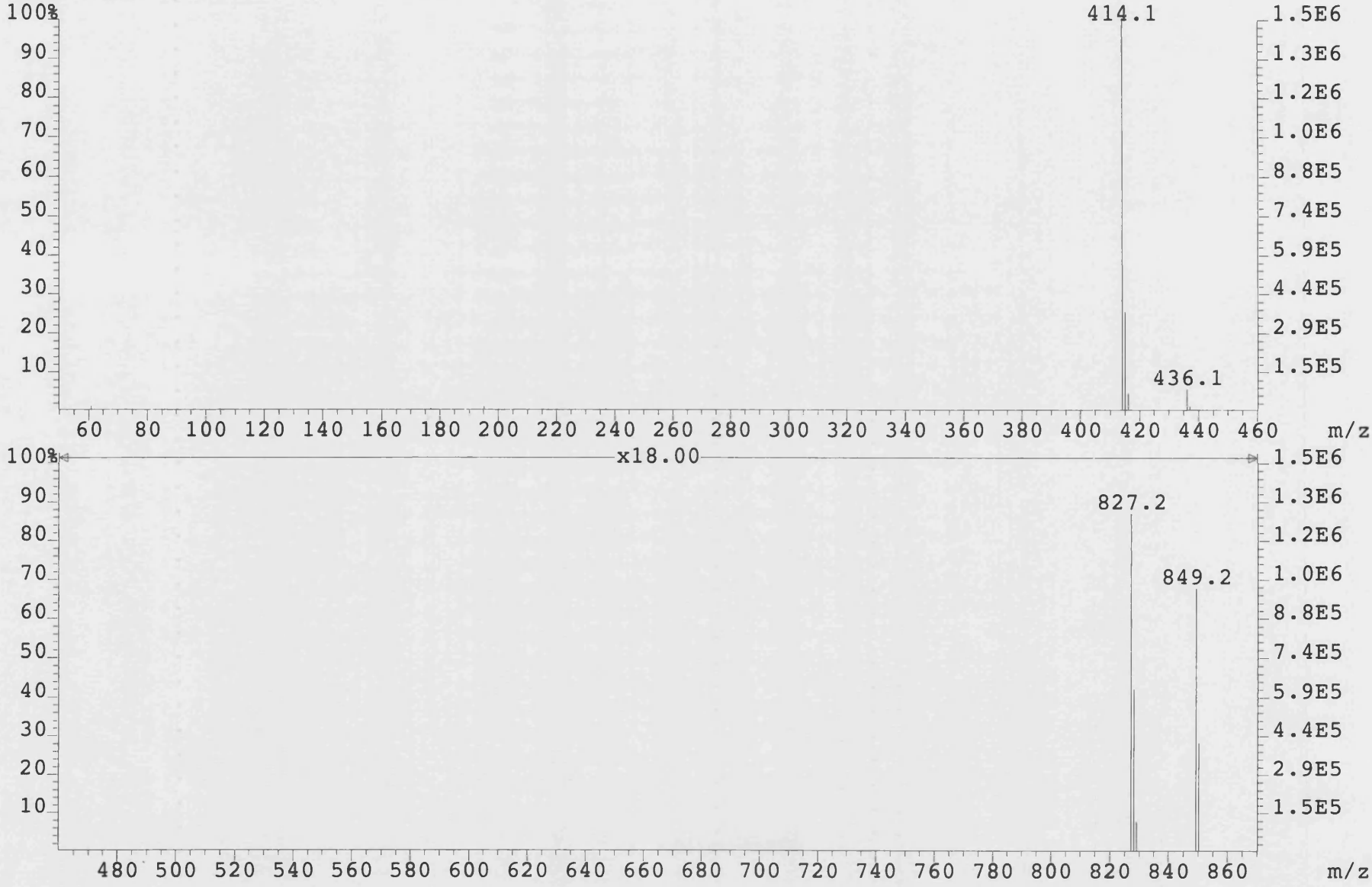

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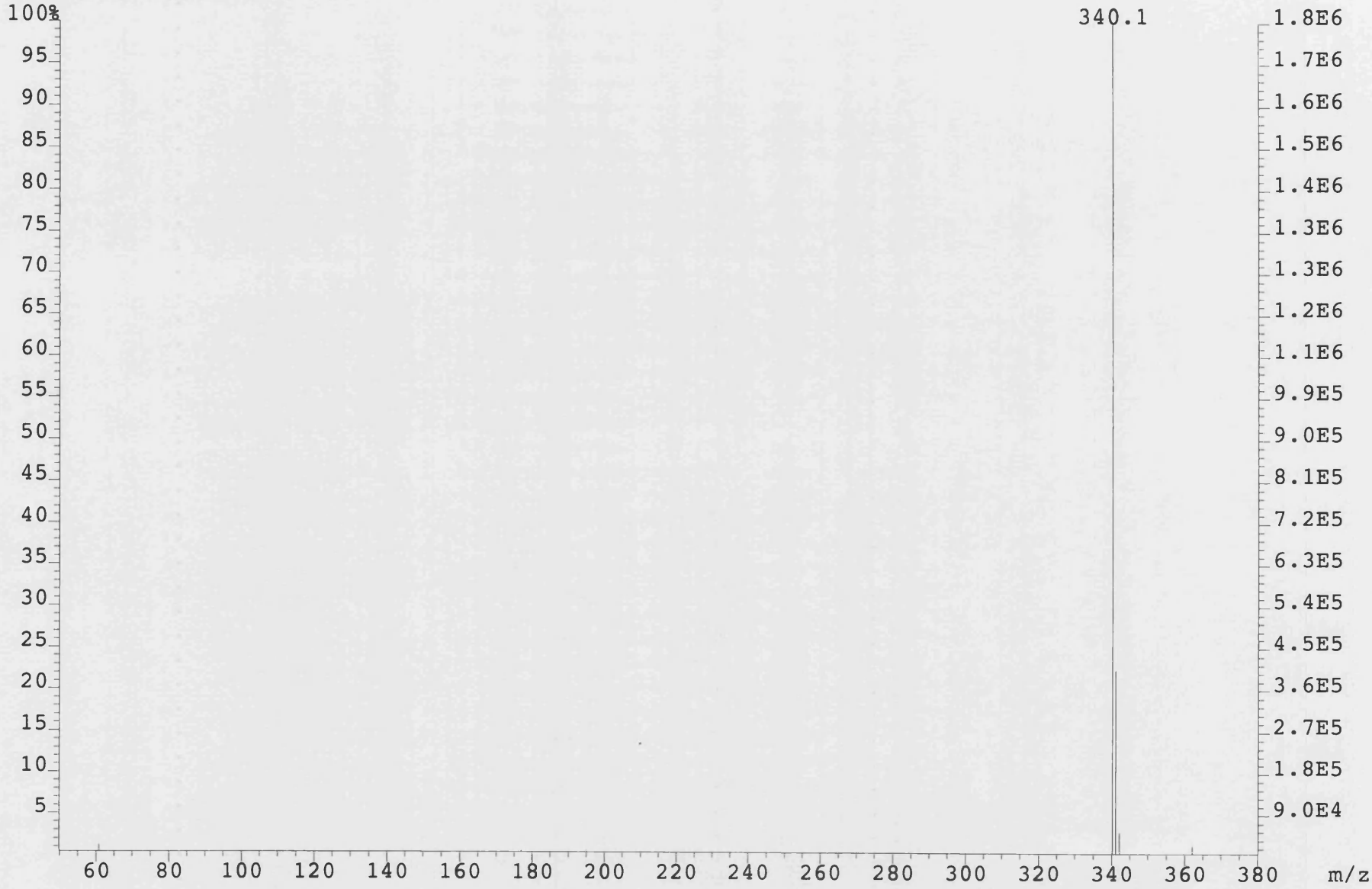

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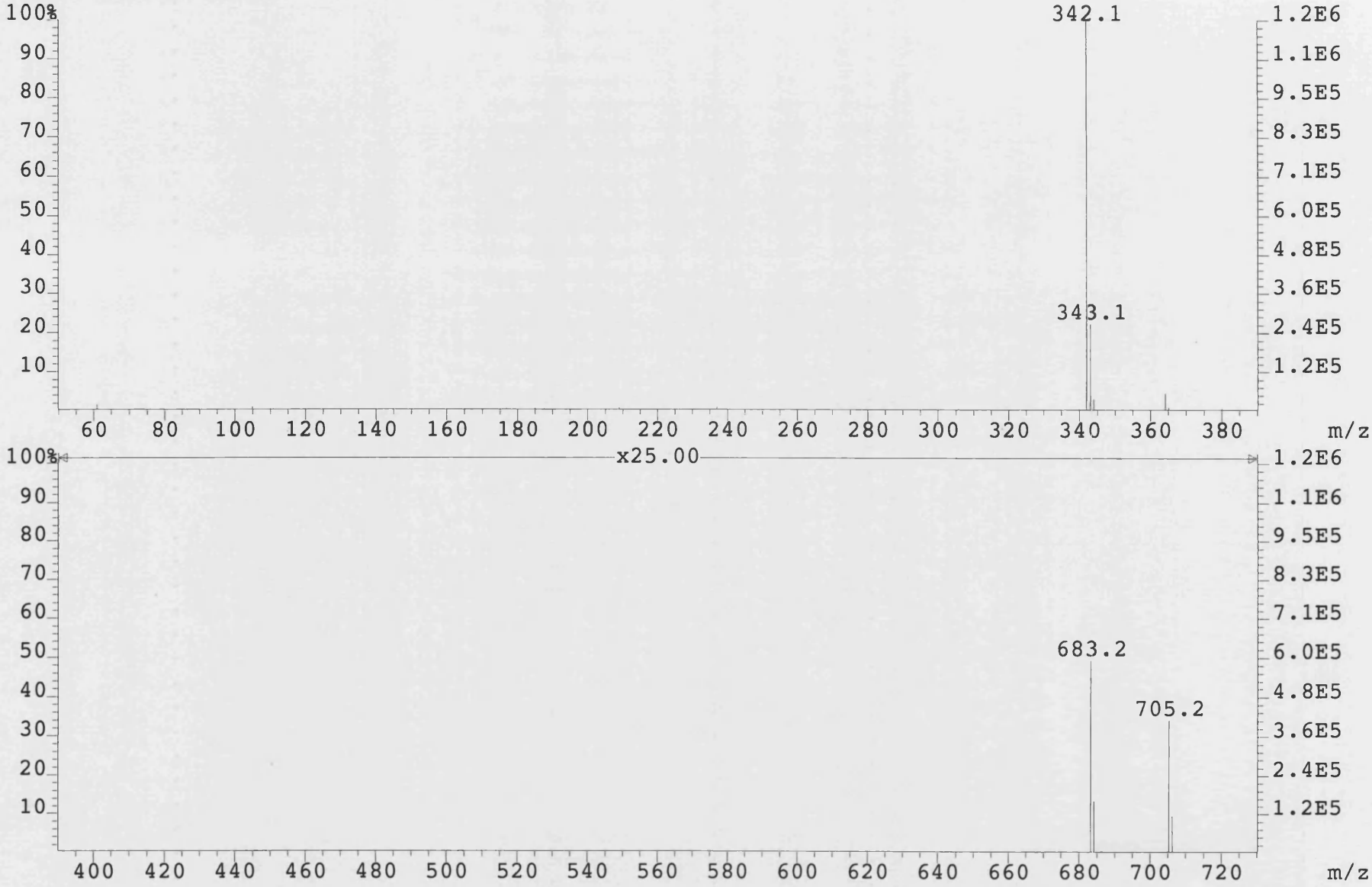

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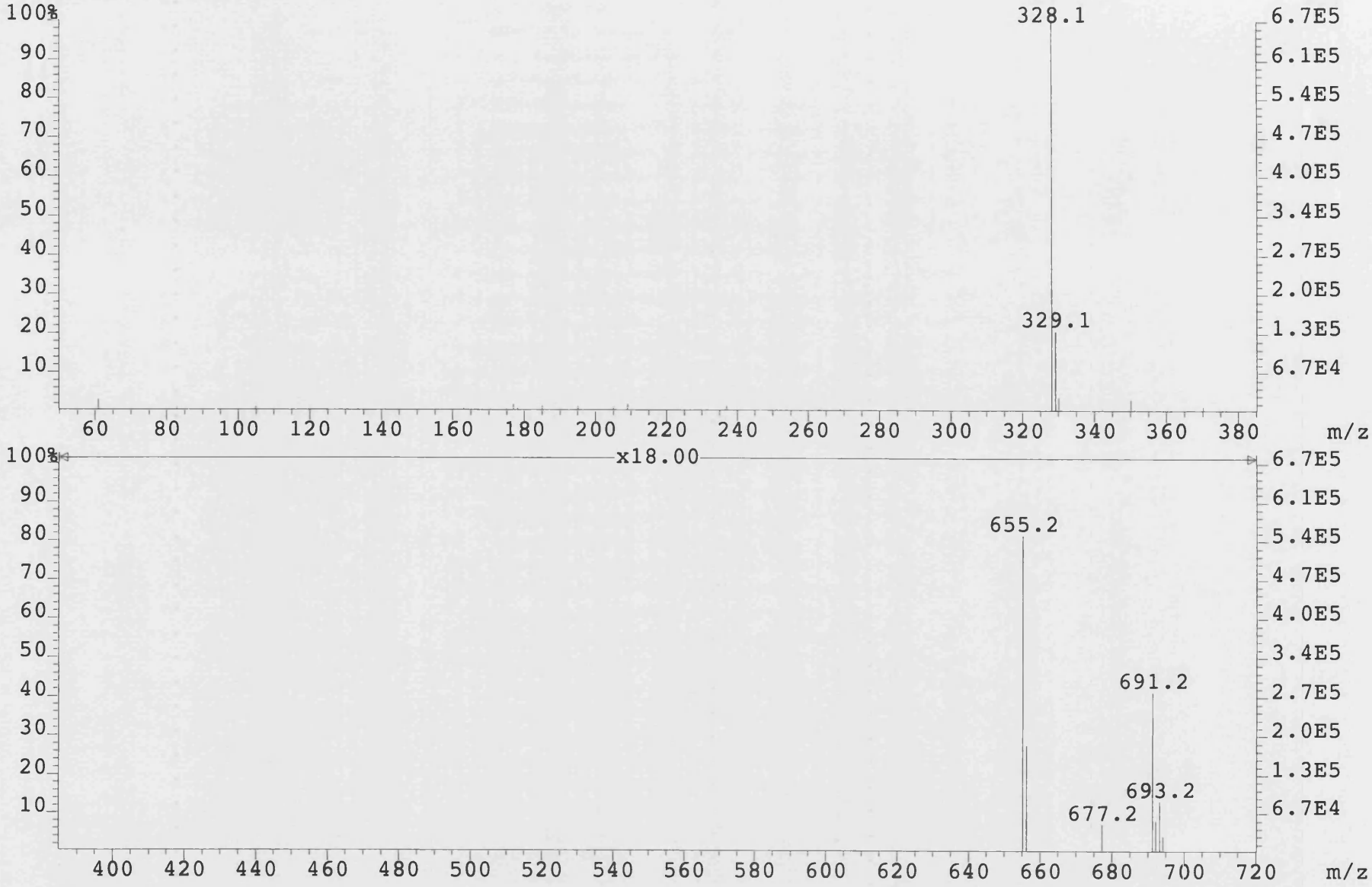

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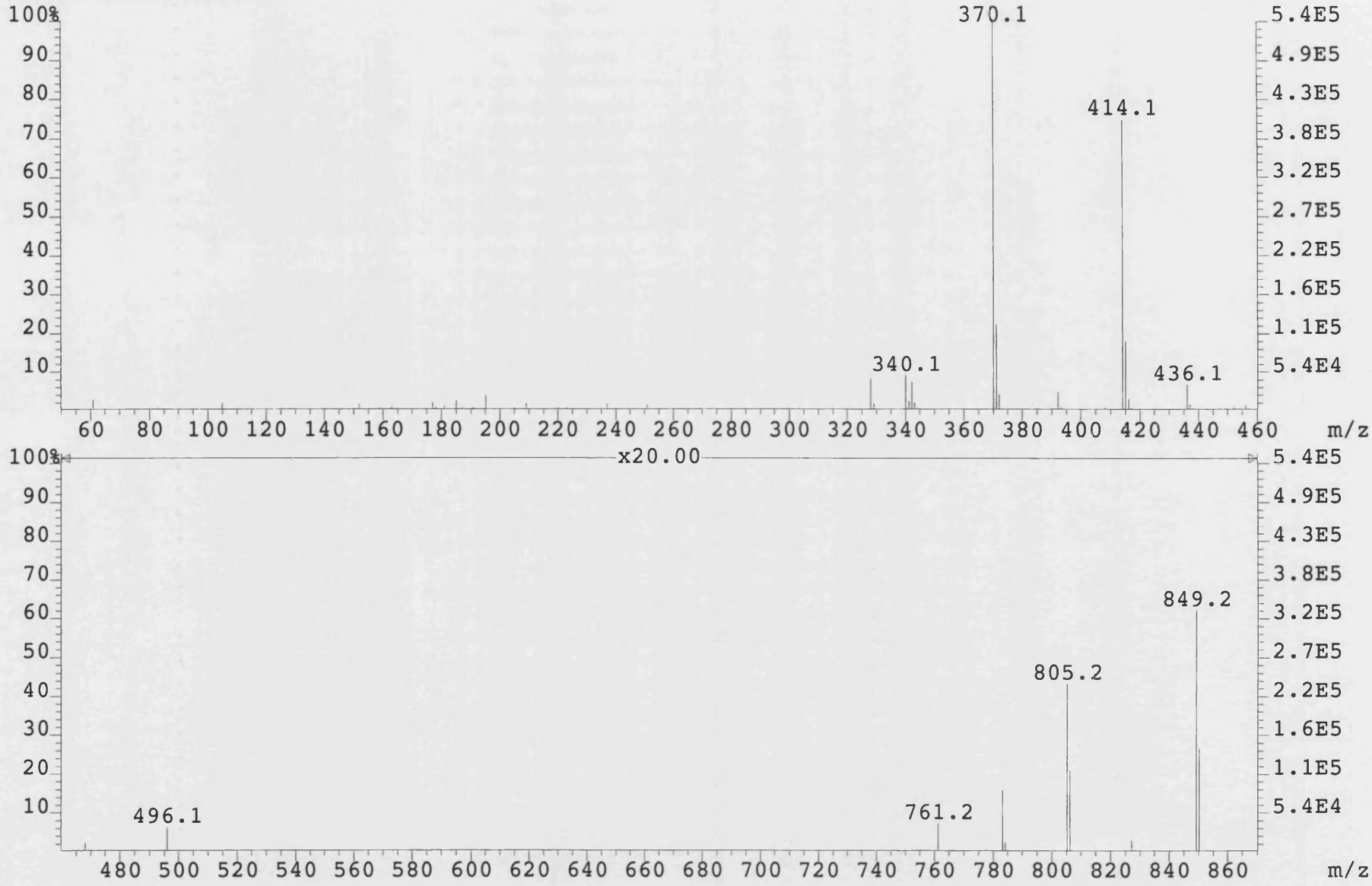

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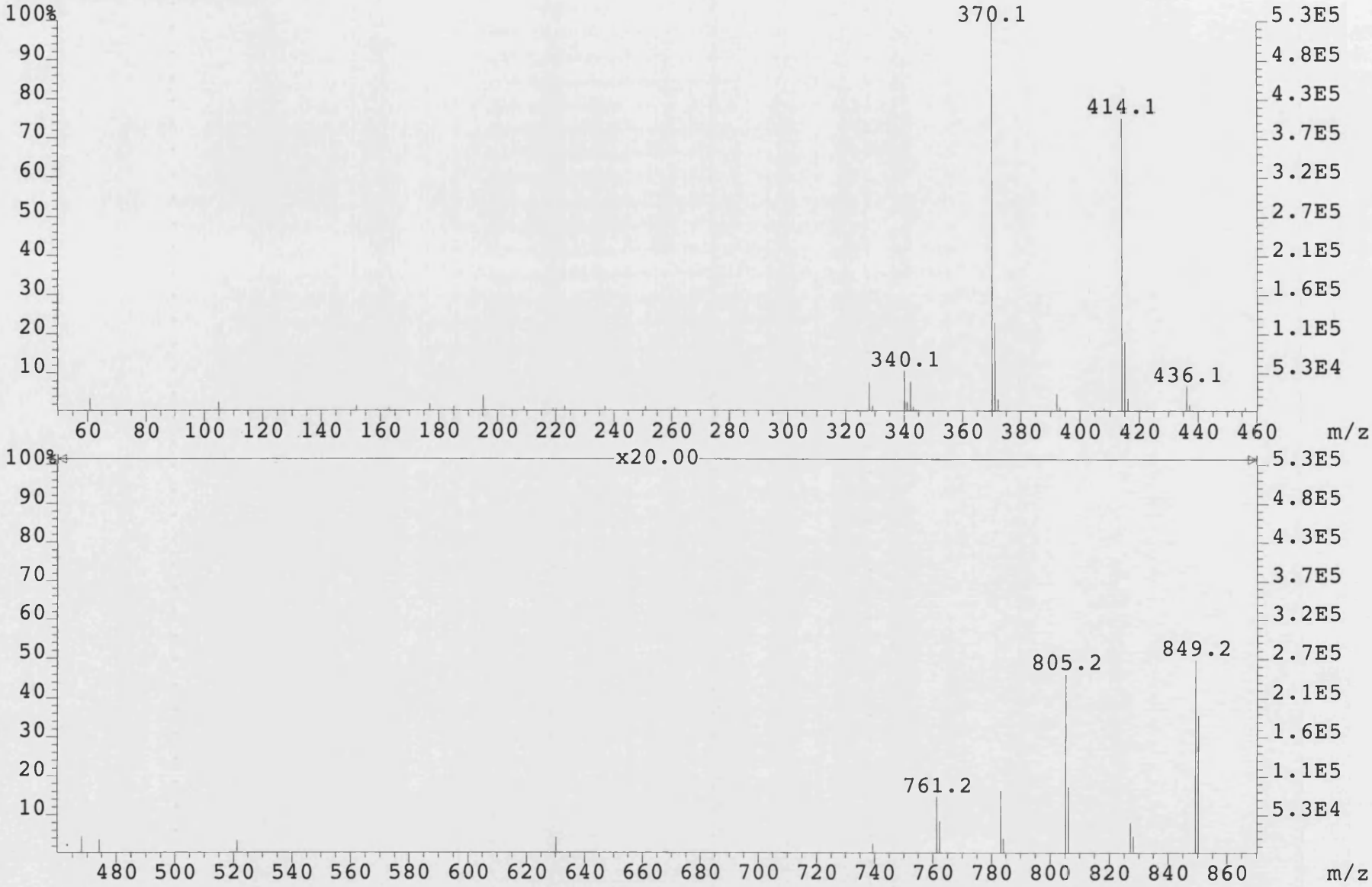

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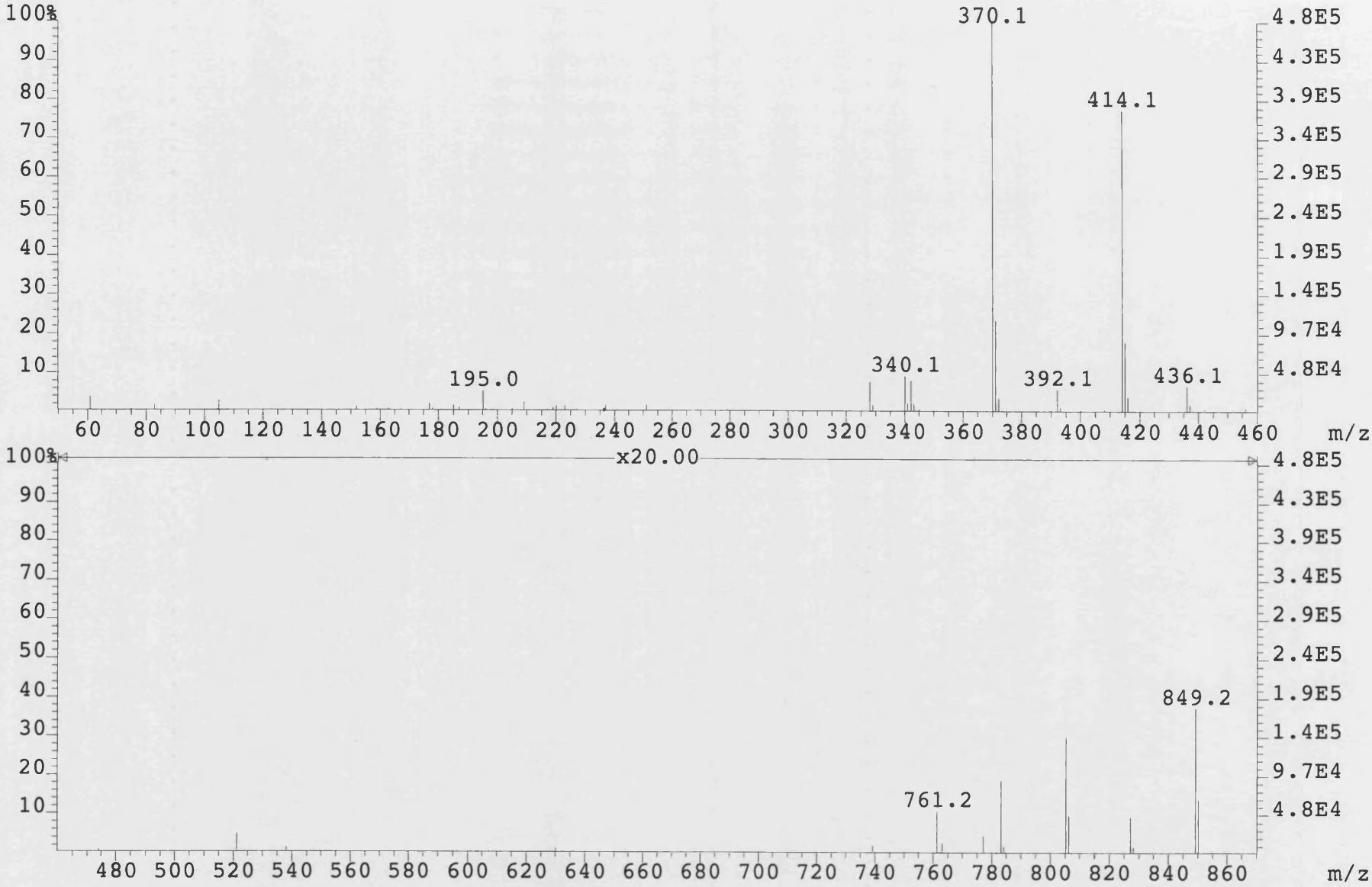


Appendix 8

NMR Spectra

NMR spectra of compounds commonly found in illicit heroin mixtures

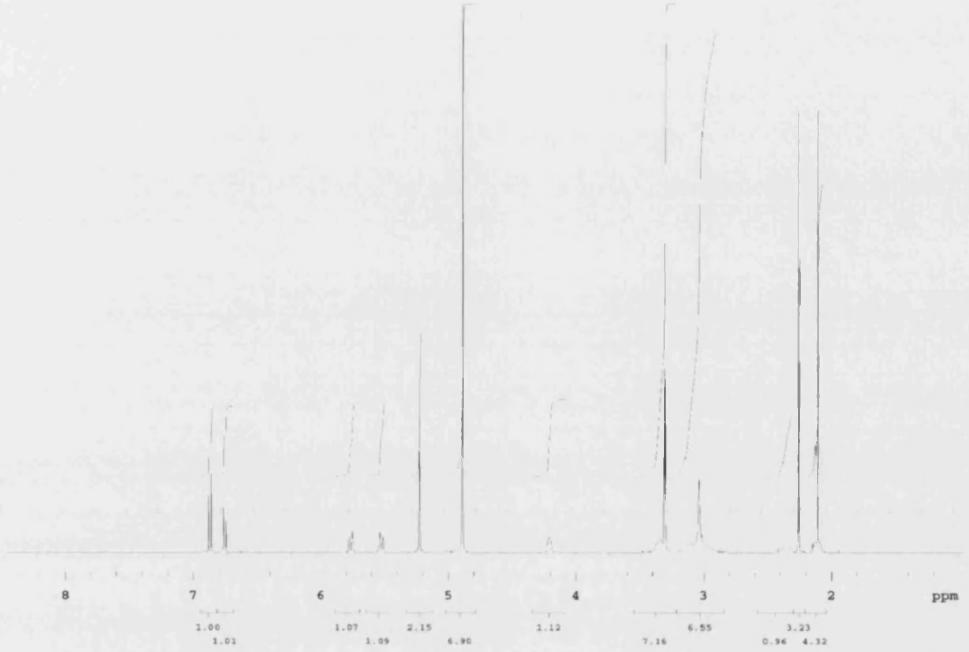


Figure 1. ^{1}H NMR spectrum (400 MHz, CD_3OD) of diamorphine

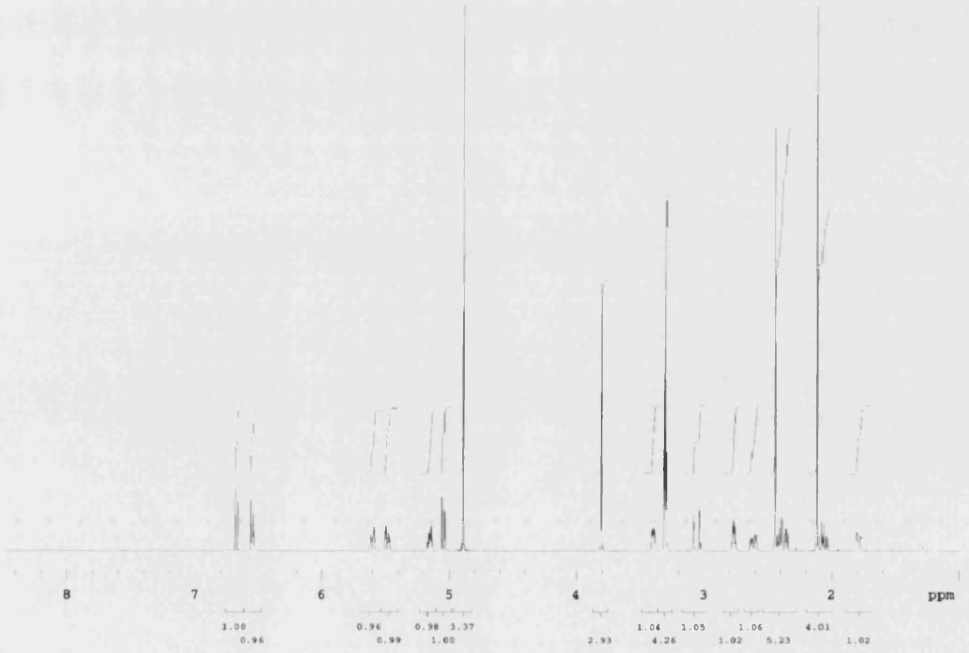


Figure 2. ^{1}H NMR spectrum (400 MHz, CD_3OD) of acetylcodeine

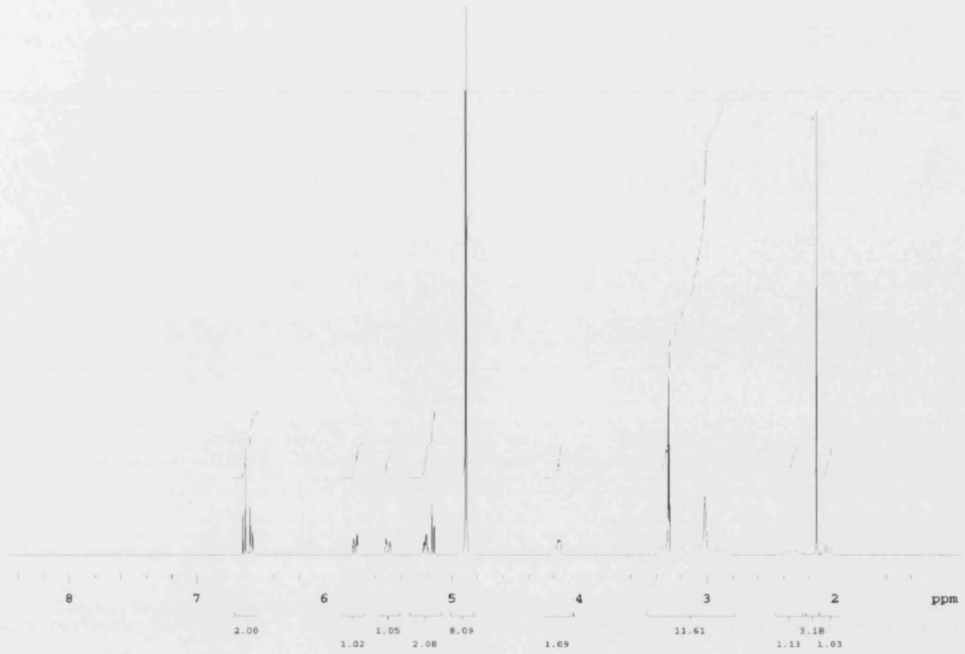


Figure 3. ^{1}H NMR spectrum (400 MHz, CD_3OD) of 6-monoacetylmorphine

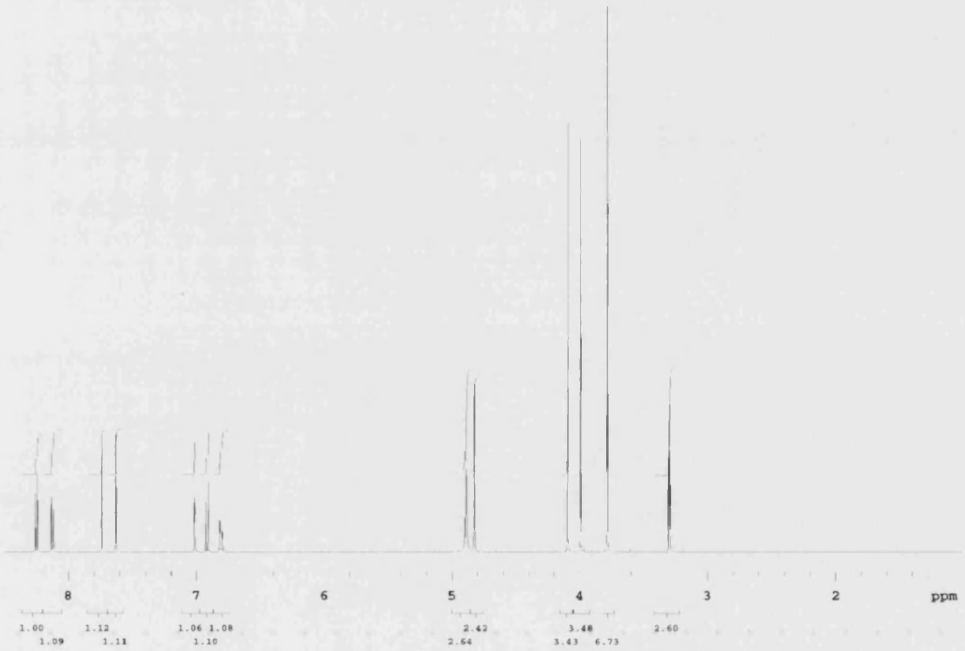


Figure 4. ^{1}H NMR spectrum (400 MHz, CD_3OD) of papaverine

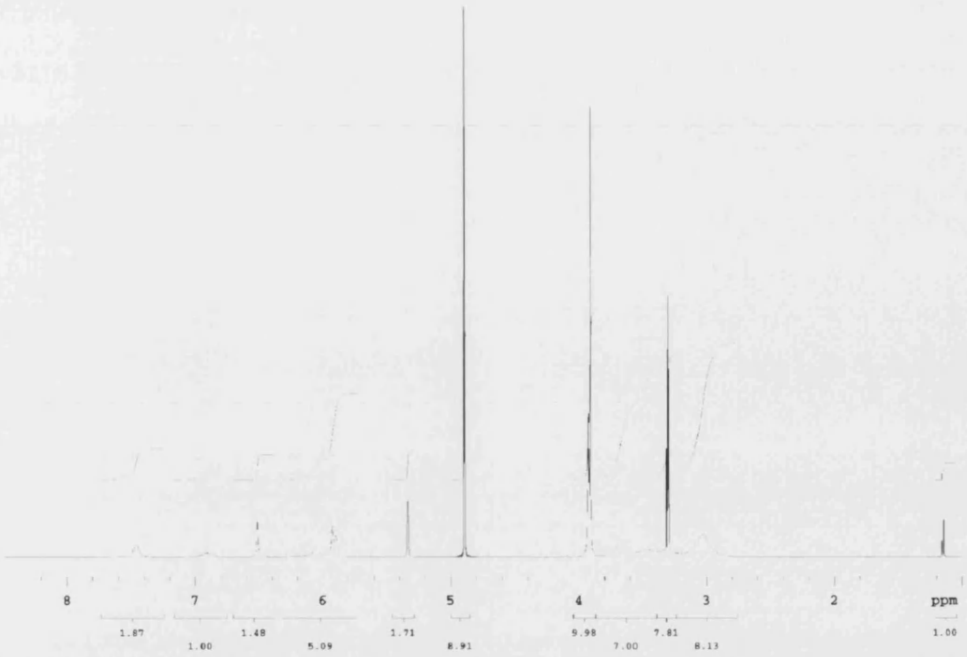


Figure 5. ^{1}H NMR spectrum (400 MHz, CD_3OD) of noscapine

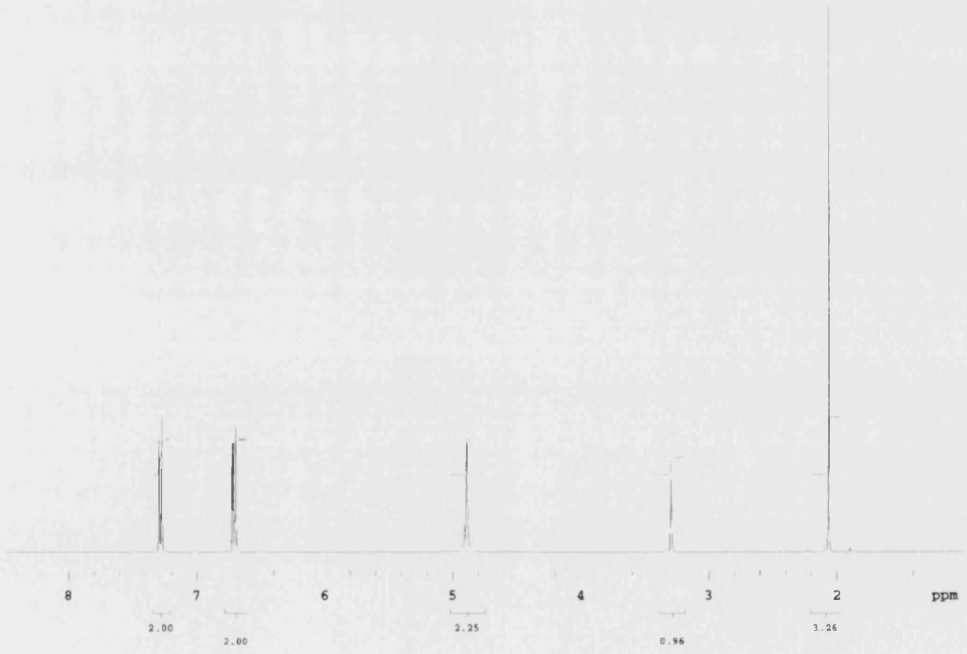


Figure 6. ^{1}H NMR spectrum (400 MHz, CD_3OD) of paracetamol

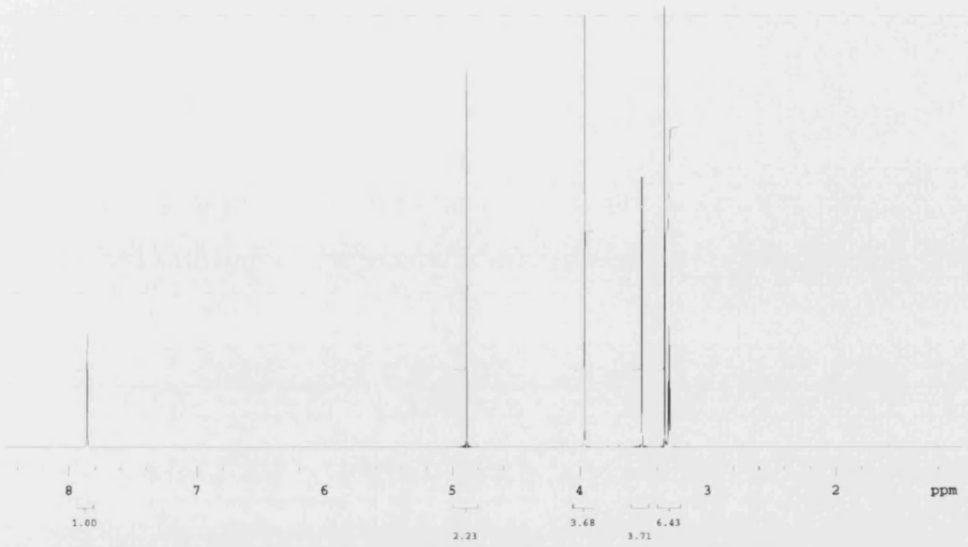


Figure 7. ^{1}H NMR spectrum (400 MHz, CD_3OD) of caffeine

Publications

IHRC 2003 Abstract

TITLE:

Heroin injection – Its preparation steps and user variations

Background/Objectives

In the UK, most heroin and crack cocaine is in an insoluble form. This requires conversion to a soluble form before it is injected. To accomplish this, users add weak acids e.g. lemon juice, vinegar and citric acid. There is little information in the literature examining the preparation steps in detail. A better understanding would inform safer injecting research and practice. This work explored user preparation procedures and investigates techniques in depth.

Methods

A novel two part method was used with needle exchange attendees at three locations. Saturation technique determined numbers. Part one was a semi-structured interview, and part two a preparation simulation using imitation drug. This was observed by the interviewer and the procedure details recorded. All steps employed, and the amount of drug, acid, water and heat used were recorded.

Results

Information from 65 IDUs was collected. The main preparation steps were similar in all interviewees. They all added the acid to the drug in the heating equipment (cooker) then added water. This was heated, and filtered before injection. However, within each step, there was variation e.g. equipment chosen, and the proportions of drug, acid and water used.

Conclusions

This work shows a great amount of variation within one common procedure. It highlights areas of injection preparation that can be targeted in harm reduction information and research. The interview procedure could be used in the future for further insight into the injecting of other drugs. The results will be used to inform laboratory work to conduct a theoretical risk assessment of injection preparation

Heroin and Crack – Injection preparation and user variations

Rhys Ponton and Jenny Scott

Clinical Pharmacy and Pharmacy Practice Research Group, The University of Bath, UK.

Introduction

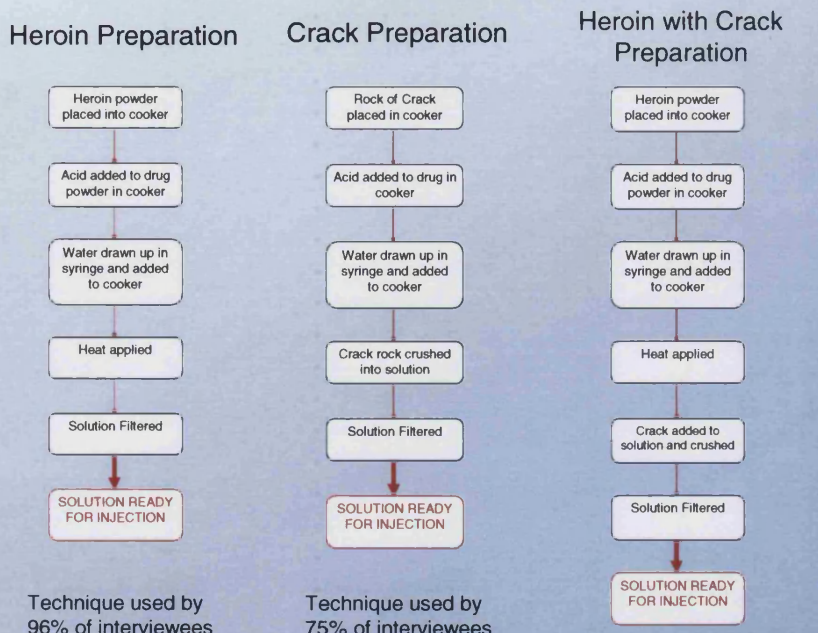
The complete process of preparing drugs for injection is poorly reported in the literature and has only been investigated by one paper to date[1].
This work investigates the process injectors use to prepare water insoluble drugs for injection and identifies aspects for further laboratory study.

Methods

Heroin and crack injectors were interviewed at three needle exchange facilities in the United Kingdom. The interview consisted of a questionnaire and the preparation of a inert 'fake' drug substance to demonstrate the methods normally used to prepare injections

Results

Most users followed the same core steps to prepare an injection



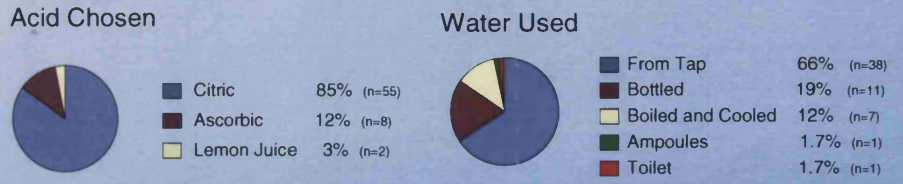
In total, 65 injectors were interviewed

Variations

Many of the interviewees used different equipment and materials to perform each step of the procedure.

Injectors use various acidic substances to help dissolve the drug. The risks of injecting these acids is unknown, and is an identified area for further study to quantify the risks.

The source of the water used by injectors is important with regard to its cleanliness and possible bacterial and/or pyrogenic content. This is a further area for laboratory study.



Conclusions

This work demonstrates that there are core steps in preparing an injection that varies little between most injectors interviewed. This is important information for harm reduction workers who need to understand the techniques injectors use so they are able to advise users effectively.

The injection preparation techniques learned in these interviews will be used to produce injections under laboratory conditions. These will then be analysed and the risks they pose quantified. The final outcome will be an understanding of the relative risk and safety of the common acids and water sources used.

Reference

Scott J, Winfield A, Kennedy E, Bond C. Laboratory study of the effects of citric and ascorbic acids on injections prepared with brown heroin. *Int.J.Drug Policy* 2000;**11**:417-22

Contact: R.Ponton@bath.ac.uk

IHRC 2005 Abstract

The microbiology of prepared heroin injections

Rhys Ponton, Jenny Scott

Background/Objectives

Sterility is a critical property of injections. Injecting by-passes the defences of the body, therefore any organisms either viruses, bacteria or fungi, present in injections can gain uninhibited systemic access, potentially causing complications. This work investigated the presence of bacteria and fungi in illicit heroin samples, and then identified the types of organisms that were still viable after the preparation of the drug sample for injection.

Methods

Heroin injections were prepared in the manner of illicit drug injectors as established in our previous work. Using a method adapted from McLaughlin et al. these injections were then prepared and cultured to investigate the growth of any viable organisms. Heroin samples were also spiked with cultures and the prepared injections tested for growth of these organisms.

This work was part of a greater study into the effects of using acids in the preparation of drugs for injection, therefore this method involves the use of acids in the injection preparation.

Results

Samples of the illicit drug material supplied by the UK Police showed growth of organisms after broth inoculation. The organisms were identified, and the risk of infection assessed. Changes to the preparation method, including varying amounts of acid and altering heating time assessed the ability to reduce micro-organisms within prepared injections.

Conclusions

The injection of these bacteria poses a potential risk, but the use of acids and heat during the preparation could potentially lower this risk. The effect of the acid and the heat on the injection and the injector however also need to be considered.

The Microbiology of Prepared Heroin Injections

Rhys Ponton and Jenny Scott

Clinical Pharmacy and Pharmacy Practice Research Group, The University of Bath, UK.

UNIVERSITY OF BATH

Introduction

The spread of blood borne viruses through the injection of illicit drugs often overshadows the effects of infections caused by bacteria and fungi. These infections can cause severe morbidity and mortality, and unlike viral infections, they do not require the presence of other injectors to occur. These infections can be transmitted from injection materials (drug, acidifier or water), dirty equipment, the environment, or the users themselves- for instance, from their skin.

The outbreak of *Clostridium novyi* infections in the UK and Ireland in 2000 was the result of drug material contaminated with this bacterium.

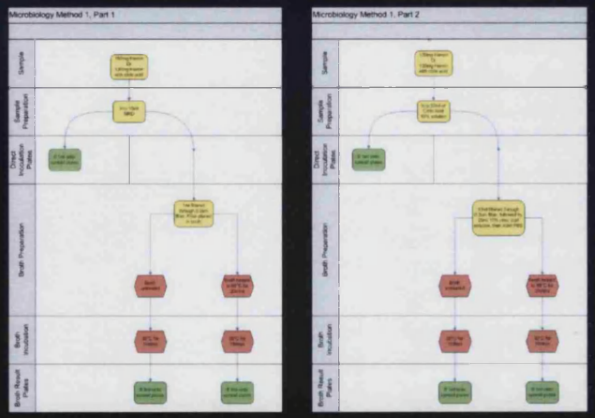
Methods

To assess the bacterial and fungal content of heroin samples, McLauchlin et al.[1] devised a microbiological culture method that would isolate a wide range of organisms. This method proved useful in identifying organisms present within samples, but did little to explain the actual risk they pose. This project repeated the work, but used prepared injections as the samples. Injections were prepared in the manner investigated through previous work[2].

Further work was conducted that involved the introduction of known potentially harmful organisms to samples, and then assessed their ability to survive the preparation process.

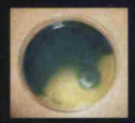
The culture method involved two main parts- the first studied aliquots of raw sample (including drug), the second involved a procedure to remove heroin from the samples on the basis that it inhibits microbial growth. To culture any organisms present, eight different agar plates were inoculated and incubated under different conditions to encourage growth.

The flow charts on the right show the steps used to prepare samples for culture.



Results

Examples of agar plate results:



The plate on the left shows the colour change produced by the growth of *Bacillus cereus*

The plate on the left shows the organisms produced on culture of the heroin sample

The culture of unprepared heroin samples demonstrated that they were unsterile. The samples contained at least three different organisms.

Cultures of heroin prepared for injection resulted in no growth.

Additionally, no growth was seen from the filters used in the preparation after inoculation into nutrient broth and incubation.

Injections inoculated with *Staphylococcus aureus* and *Bacillus cereus* before the heating stage showed no growth of these organisms after preparation.

'Injections' prepared with acid and heating, but without drug material, resulted in growth of the inoculated organisms.

Controls to ensure growth under the incubation conditions were conducted at all times.

Conclusions

The absence of growth from cultures of injections containing drug indicates that the presence of heroin during the preparation neutralises *S. Aureus* and *B. cereus*. This is of importance firstly, as McLauchlin et al. found *B. cereus* to be the most prevalent organism within their samples, and secondly, as both organisms are pathogenic.

Further Work

Investigation of the organisms present in illicit drug material and any that can survive the preparation process is warranted. This would apply to all drugs, but particularly those that are commonly reported to be produced in outside environments, where they are likely to have come into contact with soils and animals. Such work would ultimately result in better medical treatment of injectors presenting with these infections.

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ORIGINAL ARTICLE

Injection preparation processes used by heroin and crack cocaine injectors

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Abstract

Background In the UK, most heroin and crack cocaine is not soluble in water alone. They require conversion to a soluble form before they can be injected. To accomplish this, users add weak acids, for example citric acid, lemon juice or vinegar. There is little information in the literature examining the preparation steps in detail. A better understanding would inform safer injecting research and practice.

Aim To investigate the preparation of insoluble illicit drugs for injection with identification of the common procedures, materials and equipment used.

Methods A novel interview design was used. This incorporated a semi-structured questionnaire in combination with a practical demonstration of preparation of fake drug material. The preparation steps, materials and equipment used were recorded.

Results Sixty-five injectors were interviewed at three needle exchanges. The preparation of heroin, crack cocaine and 'speedball' injections was characterized. The steps to prepare injections were similar in the majority of cases. Each participant used acid in the preparation. Heroin and 'speedball' injections were heated; crack injections were not. Each injection was filtered. The equipment chosen and the proportions of drug, acid and water used varied.

Conclusions This work indicates that the basic steps used by the sample of injectors were similar, but the details within each step varied. It highlights areas of injection preparation that can be targeted in harm reduction information and research. Further work could determine the prevalence of these techniques.

Keywords: *Citric acid, injecting paraphernalia, heroin, drug preparation.*

Introduction

Illicit drug injectors prepare injections using items known as 'injecting paraphernalia'. Items used include water to dissolve the drug, acids (e.g. citric acid) to convert insoluble base drugs into soluble forms, and filters to remove insoluble particles from the prepared solution. The processes and equipment used by illicit drug injectors to prepare their injections has not been systematically studied and reported in the literature. Most information takes the form of descriptions in harm reduction leaflets (HIT 2001, Lifeline 2001) and drugs worker training materials (Derricott et al. 1999).

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Some published studies have included information on aspects of the preparation process but have not systematically examined the whole process from selection of starting materials to finished 'product', i.e. the injection. In 2000, Gaskin *et al.* interviewed injectors about their sharing of water and filters, recording the sources of water and type of filters used (Gaskin et al. 2000). Within their cohort, they found that the most popular water used to produce injections was tap water, and the most popular filter material derived from filter-tip cigarettes. Previous work by Scott (Scott et al. 1997) has also investigated the types of filters used by injectors. In addition to this work in the UK, work carried out in the USA by Koester et al. (1990) has shown that injectors there also use cigarette filter material as well as cotton wool to filter injections. This work also noted the use of spoons or bottle caps as the main forms of 'bowls' for mixing and heating drugs.

The use of acids by injecting users of heroin has been documented since the mid-1980s (Huizer 1987), after the emergence of the base form of heroin on the illicit market (Strang et al. 1997), but it is unclear how users preparing it for injection came to start using acids (Strang et al. 2001). Along with the UK, the base form of heroin is also found in Holland (Huizer 1987), Spain, and Denmark (Kaa 1994), and the use of acids has been recorded in Spain (Page and Fraile 1999) and Australia (Gallo et al. 1985). Previous work in this area (Scott et al. 2000, Strang et al. 2001) has recorded which acids injectors use, and with which drug. The types and the forms of the drugs used by injectors were correlated with the preparation technique with regard to acid requirement, and it was seen that the use of acid was dependent on the solubility of the form of the drug used. However, neither the way the acids were used nor how they fit into the whole procedure was investigated.

There are several benefits to systematically examining the preparation process. Information on the choice and source of equipment (e.g. choice of acids used to dissolve insoluble base drugs) could inform the delivery of harm reduction information and add research support to anecdotal reports that already exist. Such information would also allow preparation methods to be copied in the laboratory so their impact on health and risk could be studied in detail. The aim of this study was to report the process of injection preparation used by a nonrandomly selected sample of injectors and to describe the types of paraphernalia used.

Methods

To investigate the injection preparation process, current injectors would need to be interviewed. The process that injectors use to prepare an injection is followed regularly and they know almost instinctively how to complete it. This interview intended to make the participants consider the steps and the equipment required to perform the procedure. In order to characterize the method in detail, it was decided to devise a situation where the participant could demonstrate the process they follow using a fake drug substance. During the procedure, they would have access to any equipment and materials required, but they would have to request them from the interviewer. This impelled the participants to recall each detail of their usual process and avoided distortion of the results caused by prompting.

The data-collection process consisted of two parts. First, a semi-structured interview gathered data on demographics, drug use and injecting histories. It also explored in detail the participants' use of acids for dissolving drugs. The responses were recorded in writing by the interviewer on a prepared form.

The second section of the data-collection process involved the participant demonstrating their usual preparation technique using the inert chemical substance in place of a drug. They were asked to prepare an injection of the inert chemical as if it were their real drug of choice. The inert substance used was derived from heated and ground paracetamol tablets, modified to give visual characteristics similar to illicit heroin base and crack cocaine.

To document the process, the options of audio recording, video recording and visual assessment (with note taking) were considered. As the procedure was of most importance, audiotape would be of little value. The option of video recording the process was discarded to prevent recruitment problems caused by concerns about confidentiality and to prevent any effects of having a camera present, for instance anxiety of being filmed or 'performing' for the camera. After careful consideration, it was decided that the preparation technique would be observed by the researcher, and recorded on a checklist.

The recording checklist included selectable options described in safer injecting leaflets and the literature (Derricott et al. 1999, HIT 2001, Scott et al. 2000) and space for noting unpredicted actions. Paraphernalia for the preparation the process were available upon request from the interviewer. To prevent bias in prompting the users, the equipment was kept out of sight in a box until requested. Table 1 lists all the equipment that was available to the participants.

The fake inert chemical substance, the citric acid and the ascorbic acid were issued in preweighed quantities. These were reweighed after use to allow the amounts used to be calculated. The amount of water used and heating time, if used, were also recorded.

Table I. Materials used for interview procedures.

'Drug'	Inert substance with similar physiochemical properties to heroin or crack cocaine. This was made from paracetamol tablets.
Acids	Ascorbic acid (500 mg in eppendorf tube)
	Citric acid (500 mg in eppendorf tube)
	Lemon juice – Jif lemon
	A real lemon
	Bottled vinegar
	Sachets of baby bottle sterilizer
	Descaler sachets
Water	The participants were asked where they usually obtained this.
Cookers	Teaspoon
	Dessert spoon
	Crimped beer bottle caps
	Empty drinks can (to enable use of bottom)
Filters	Hand rolling cigarette filters
	Filter-tip cigarettes (to enable use of cigarette filter material)
	Cotton wool
	Cotton wool buds
Heaters	Lighter
	Matches
	Night light/candle
	Pre-injection swabs (for burning if that is usual practice)
A varied selection of syringes and needles, incl.:	0.5-ml and 1-ml insulin syringes (with fitted needles 29G (0.33 mm))
	2.5ml and 5ml syringes
	Orange (25G (0.5 mm)), blue (23G (0.6 mm)) and green (21G (0.8 mm)) needle

Participants were asked to follow their usual procedure without any deviation. They were not interrupted during the process unless absolutely necessary, for example if they performed a unique action and clarification was required. Interruption was kept to a minimum to prevent distracting the participant, which could possibly induce errors. Once the preparation was complete, all used equipment was collected and the prepared fake injection disposed of.

Needle exchanges in the city of Bristol and the town of Hereford, England, were used as interview locations. Piloting of the methods was undertaken at a needle exchange in Bath. Prior to commencing work at each facility, approval for the study was gained from the Local Research Ethics Committee for the area.

Users of the exchanges were made aware of the study through posters and leaflets that outlined the study and what would be required of participants. Users were also informed of the work during the needle-exchange process and, if willing, introduced to the researcher (RP) by the exchange workers. The researcher explained the study, confirming that the potential participant was an injector of either heroin, crack cocaine, or both. The researcher stated that participation was voluntary and would not affect the service received from the exchange agency. He also eliminated anyone who did not prepare their own injections, as it was considered that they may not be able to provide detailed information. The participant was given the opportunity to ask questions. The researcher then obtained the verbal consent of willing participants. Names of participants were not asked or recorded in keeping with the practice of the exchange agencies. Therefore, consent was obtained verbally from each participant as asking them to sign a consent form would breech this anonymity. The interviews were conducted in a private room with only the researcher and the participant present. All data were collected by the same researcher for consistency.

During the consenting procedure, participants were warned that the preparation demonstration could possibly induce cravings, and, if this was felt to be a problem, then a semi-structured interview could be conducted instead. Participants were informed that they could leave the room at any point without giving a reason. At the close of the demonstration, participants were thanked with a £10 gift voucher for their time and participation. This reward was not made known during the recruitment or consenting procedures.

Results

Sixty-five injectors participated in the study: two people in Bath, 28 in Hereford and 35 in Bristol. All participants chose to complete the interview and the practical demonstration.

Of the participants, 80% ($n=52$) were male, 20% ($n=13$) were female (ratio 4:1). The mean age of the male participants was 29.1 years (range 20–46 years); of the females it was 25.8 years (range 18–38 years). Thirty-four per cent ($n=22$) of the participants were under 25 years old; 66 % ($n=43$) were 25 years old or older. The length of time injected for ranged from 3 months to 18 years, the mean being 6.38 years.

All 65 participants had injected heroin ever. Forty-five of the participants had injected crack ever, and 39 of the participants had injected the combination of the two (as a 'speedball').

A wide range of other drugs had been injected by the participants, including: amphetamine ($n=46$), cocaine powder ($n=24$), diazepam (including Valium® ampoules;

$n=10$), temazepam ($n=11$), 'ecstasy'/MDMA ($n=13$), Diconal® (dipipanone with cyclizine tablets; $n=10$), Palfium® (dextromoramide tablets; $n=8$), LSD ($n=5$), dihydrocodeine ($n=4$) and cyclizine ($n=4$).

All 65 participants stated they used an acid to prepare their injections. Thirty-four (52%) stated that they preferred to use citric acid. The remaining 31 (48%) stated their preferred acid was ascorbic acid. However, the acid used by the participants on a day-to-day basis was different from the preferred stated acid. Fifty-six (86%) of the participants usually used citric acid, with only five (8%) using ascorbic acid. Two participants said that they used either lemon juice or citric acid, each on an equal basis. The remaining two said they had no preference for what they used or used 'whatever was available'.

Alternative substances that had been used as acidifiers included vinegar, appliance descaler preparations, vitamin C tablets, fruit juices including orange, grapefruit and lime, and the coatings from 'fizzy' sweets, which contain citric acid.

The preparation of heroin was demonstrated by 47 participants, the preparation of crack cocaine by eight participants and the preparation of 'speedballs' by 10 participants. The preparation of heroin was demonstrated at all three sites, but the injection of crack cocaine and 'speedballs' was confined to Bristol.

Steps of the preparation process

Common steps were identified that formed the basic technique used by most participants. However, within each step there was variation, for instance with the equipment used to perform it. The specific detail for each drug will now be discussed:

Heroin. Of the 47 participants who demonstrated heroin preparation, 45 (96%) followed the same five basic process steps. These are shown in Figure 1. This shows the drug being mixed with the acid first, and then the addition of the water. The two participants who did not follow these steps demonstrated variation in the order they mixed the drug, the acid and the water. One of these added the water to the drug powder, before adding the acid powder. The second choose to measure out the acid powder, and then add the drug powder to it.

The equipment used by the participants refers to the paraphernalia used, including the needle/syringe, the cookers, the heat sources and the filters. 'Materials' refers to the water (defined by its source) and the acid used. For the heroin injections, the choice of acid used was the first main point that divided this group. The majority of participants chose to use citric acid ($n=37$, 79%), eight (17%) chose ascorbic acid, and two (4%) chose lemon juice. Water was the second dividing factor. Thirty (64%) used water from the tap, seven (15%) used water from bottles (including bottled mineral waters and tap water stored in a bottle), six (13%) used boiled water and one (2%) stated using sterile water from ampoules (source of these unknown). Data on water are missing for three participants.

'Cookers' used included teaspoons, dessertspoons and the bottom of used drinks cans. Forty-five participants (96%) chose to use a spoon of either size; the other two used the bottom of a drinks can. To filter their injections, 29 (62%) of the participants used material from filters made for hand-rolled cigarettes, 14 (30%) used material from filter-tip cigarettes and four (8%) used cotton wool.

Tables 2 and 3 depict the quantities of the drug substitute, the acid powder and the water used during the demonstrations. To remove any influence that the insolubility of

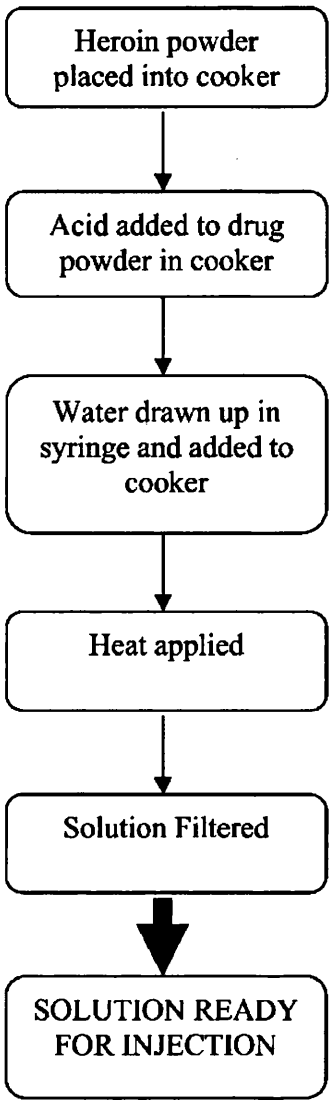

Figure 1. Steps shown by 96% ($n=45$) of heroin injectors as the usual method used to prepare heroin injections.

the drug substitute may have exhibited, the data from any participants were removed from the results before the below values were calculated. Therefore, the figures indicate the quantity of acid and water the participants would use for a given volume of drug.

The participants were asked during the process how much the drug substitute they were using would be sold for on the illicit market. For the citric users, the fake drug quantities

Table II. Quantities used in heroin injection preparation using citric acid ($n=28$, corrected to remove participants who added extra acid during or after heating, and those who spilled powder from the tubes during the interview).

	Heroin substitute (g)	Citric acid (g)	Water (ml)
Mean	0.122	0.079	0.85
Min.	0.053 (quoted as £10 in value)	0.018	0.50
Max.	0.284 (quoted as £10 in value)	0.284	1.50

Table III. Quantities used in heroin injection preparation using ascorbic acid ($n=7$, corrected to remove participants who added extra acid during or after heating).

	Heroin substitute (g)	Ascorbic acid (g)	Water (ml)
Mean	0.169	0.175	0.91ml
Min.	0.070 (quoted as £10 in value)	0.194	0.45
Max.	0.287 (quoted as £20 to £25 in value)	0.335	2.20

used ranged from £5 to £40 in value. The mean value was £12.71, and the most commonly used amount was the amount of a £10 'bag', which was used by 15 of these 28 participants.

Of the seven participants who used ascorbic acid, the value of the fake drug used ranged from £10 to £25–30. The mean quantity used was £17.14, with three of the seven using the quantity they expected to obtain in a £10 bag.

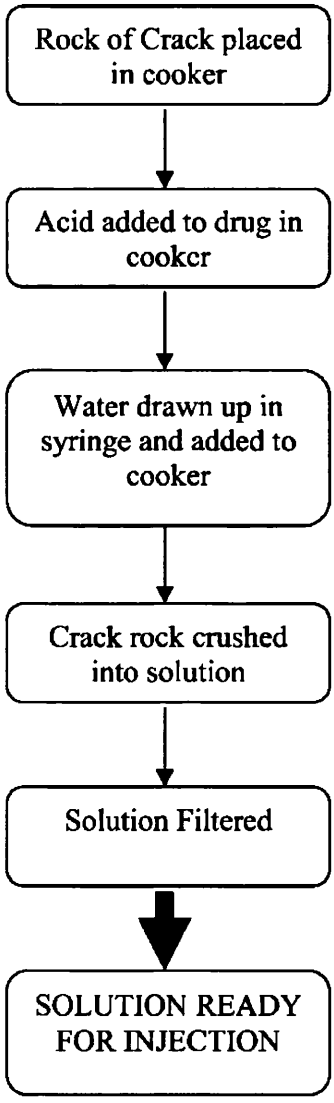

Figure 2. Steps shown by six out of eight crack cocaine injectors as the usual method used to prepare crack cocaine injections.

Crack cocaine. Eight participants demonstrated the preparation of crack cocaine for injection. Six of the eight participants followed the same order of steps, depicted in Figure 2: The other two added the acid *after* adding the water to the drug in the 'cooker':

All crack cocaine injectors ($n=8$) chose to use citric acid for their preparation. Water source varied, with three using tap water, one using 'boiled and cooled' water from the kettle, one using bottled water, and one using water obtained from a toilet. Data on water are missing for two participants. Seven used spoons as cookers; one used the bottom of a drinks can. To filter, all eight used material from filter-tip cigarettes.

The crack substitute used by the participants would range from £4 to £10 in value on the illicit market. The mean value was calculated as £8.86, with five of the seven stating the amount they used was worth £10. Table 4 details the quantities of the materials used in the crack user interviews.

Table IV. Quantities used in Crack injection preparation demonstrations ($n=7$, corrected to remove participants who added extra acid during or after heating).

	Crack substitute (g)	Citric acid (g)	Water (ml)
Mean	0.099	0.069	0.58
Min.	0.039 (quoted as £4 in value)	0.025	0.40
Max.	0.155 (quoted as £10 in value)	0.128	0.80

Heroin+crack cocaine: 'speedball'. Ten participants demonstrated the preparation of 'speedballs'. All 10 followed the same procedure for preparing their injections; a procedure that was seen to be an amalgamation of the heroin and the crack preparation processes (see Figure 3). First, the heroin was prepared as in Figure 1, then the crack cocaine was added to the heroin solution and crushed. This was then filtered prior to injection.

All the speedball injectors who participated used citric acid for their preparation. Five used tap water and three used bottled water. Data on water are missing for two participants. As cookers, eight used spoons, while the other two used the bottom of a drinks can. To filter, eight speedball injectors used material from filter-tip cigarettes, and the remaining two chose material from roll-up cigarette filters.

The quantities of drugs used in a speedball were usually of equal value for both drugs, for instance £10 of heroin, with £10 of crack. The most common combination was £10 heroin with £10 crack, which was used by five of the eight participants. The cost of heroin used by the participants ranged from £5 to £10–15 worth. The cost of crack used ranged from £5 to £20. Table 5 details the quantities of the materials used in the speedball preparations.

Table V. Quantities used in 'Speedball' injection preparation demonstrations ($n=8$, corrected to remove participants who added extra acid during or after heating).

	Heroin+crack substitute (combined weight g)	Citric acid (g)	Water (ml)
Mean	0.207	0.115	0.81
Min.	0.105 (quoted as £10 of heroin and £10 of crack in value)	0.044	0.50
Max.	0.406 (quoted as £10 to £15 of heroin and £20 of crack in value)	0.286	1.00

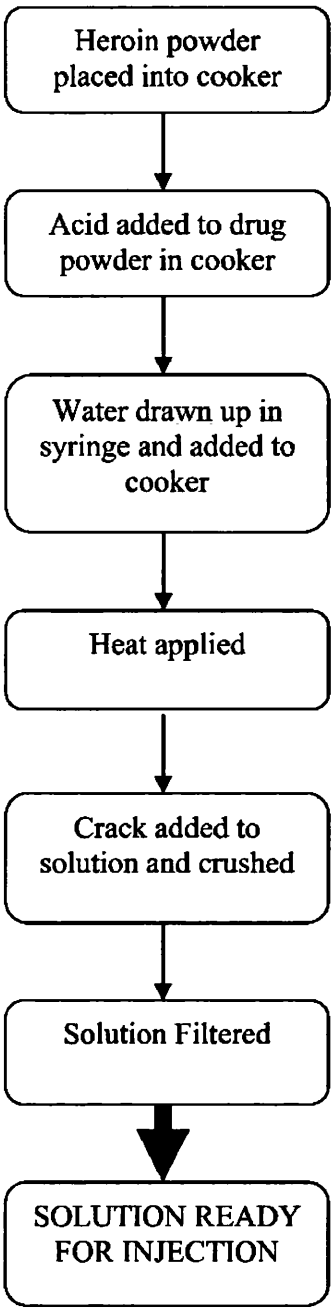

Figure 3. Steps followed by all participants ($n=10$) to prepare a 'speedball' injection.

General findings

At all three locations, each participant (except one) used only a portion of the filter specified as used. Strips were torn vertically from the roll-up cigarette filters for use. Filter-tip cigarette filter material was either pulled out of the end for use, or a horizontal slice was cut off the filter with a pair of scissors. Cotton wool was used in small balls measuring approximately 3 mm in diameter.

Although not specifically explored, it was observed that choice of acid, filters and syringes/needles used by the participants appeared to be influenced by the availability of these items at the needle exchange. The Hereford needle exchange supplies both citric

and ascorbic acids to its users, the Bath needle exchange only supplies citric acid, whereas the Bristol exchange supplies neither. The Hereford and Bath exchanges also supply filters, but Bristol did not at the time of the interviews. Where items were available at the exchange, the users were usually seen to choose to use it rather than an alternative obtained elsewhere. Only one user at the exchanges where acids were issued stated they obtained their acid elsewhere. Similarly, only two users out of 28 who attended the needle exchanges that supplied filters chose not to use those available.

Discussion

The interviews have recorded the injection preparation technique used by a non-random sample of injecting drug users. They enabled the characterization of the preparation process of heroin, crack cocaine and 'speedballs'. Out of the 65 participants, 47 demonstrated the injection of heroin, making the results for this the most reliable. The lower numbers of the crack and speedball injectors produced less reliable but nevertheless interesting results. The investigation of the preparation of these injections is a potential source of further work. The choice of three interview locations has also demonstrated the similarities, as well the differences between them. The findings of this research correlates with that of previous work, which has reported some steps of preparation procedures (HIT 2001, Scott et al. 2000), the acids used (Kinzly 1997, Scott et al. 2000, Strang et al. 2001), waters used (Gaskin et al. 2000), and the filters used (Scott et al. 1997). This work extends these articles by observing the process as it is performed and recording the complete process in detail, including all steps, materials, equipment and quantities used. The participants were not just asked questions regarding their preparation; there are, therefore, fewer reliability issues in respect of the recall of their actions.

The basic steps of the process were common amongst the majority of users. These were clearly defined and easily identified. The evolution of this sequence of steps is of interest and would be an interesting area of exploration. It is unclear why the majority of users, though recruited from two distinct locations and the pilots recruited in Bath, follow the same sequence.

The addition of the acid to the drug before the addition of the water suggests that injectors are making a judgement of how much they need to dissolve the drug before they have even tested its solubility in water. The application of heat was witnessed in all preparations involving heroin. Strang et al. (2001) found that this was dependent on the type of heroin used (brown as opposed to white), and this would correlate since the substitute heroin used in this work was brown in colour. The drug content of injections that have been heated during preparation has yet to be investigated; however, research by Clatts et al. (1999) indicates that heating injections can inactivate HIV, and Collignon and Sorrell (1983) have suggested that the heating of heroin injections can prevent systemic candidal infections.

The filtering of the injection solution was universal amongst all 65 participants. Particulate matter contained within injections can cause phlebitis; therefore, this step may have a protective role. However, the use of inappropriate material may lead to different health complications, for example from particle shedding. Filters of a high quality should be available from needle exchanges to reduce the numbers of injectors resorting to less suitable materials.

The use of acids was also common to all participants. All of them used one of the three

main types of acid available: citric acid, ascorbic acid, or lemon juice, which contains both citric and ascorbic acids. None chose to use vinegar or kettle descaler sachets that, according to anecdotal reports, are used, although participants did report having used them before. This probably indicates that they are acidifiers of 'last resort'. Lemon juice has been implicated as the cause of systemic candidal infections (Bisbe et al. 1987, Dally et al. 1983, Gallo et al. 1985, Mellinger et al. 1982) and candidal endopthalmitis (Shankland et al. 1986, Shankland and Richardson 1989); therefore its low level of use (two of the 65 participants) was encouraging and may suggest that safer injecting messages are getting through. It is also important to note that the two users who demonstrated the use of lemon juice only used it half of the time for injection preparation, and they also reported being aware of potential dangers in its use. Citric acid was the most commonly used acid (85%, $n=55$); however, the long-term health implications of its injection are still unknown. Acute effects from citric acid injection can include burning in the peripheral veins and thrombophlebitis, and these dissuade some injectors from using it.

The results for the quantities of the materials used were interesting, but there appears to be little correlation between the quantities used. There are no apparent links between the amount of drug, acid or water used. In any event, the sample sizes for the heroin-ascorbic, the crack and the 'speedball' users are too small to draw any valid conclusions.

The interview design used for this work was devised specifically for this work and has never been used before. Some limitations in this work are apparent.

Alternative demonstration designs were considered, but were dismissed, for instance the use of real drugs, including participants using their own drugs. This was discounted on numerous grounds. First, interviews were carried out on drug service premises, which, for legal and ethical reasons, forbid the preparation and consumption of illicit drugs. Second, having prepared the drug, the user would want to inject it, or receive recompense. The use of the drug would carry risks of overdose, and payment would raise legal and ethical issues. Last, taking the drug from the interviewee would have constituted an illegal supply of a controlled drug and have put the interviewer in unlawful possession.

The inability to use real drug materials may have led to differences in the procedure used, for example changes to the heating time. The addition of extra acid by the injectors during the preparation process was possibly related to the slightly higher insolubility of the fake substitute, but it gives a valuable insight into the procedure. The use of real drug materials would probably have caused technical and legal issues that would probably prove complicated to resolve, and therefore this technique, even with its limitations, has proved workable.

The technique of recording the process using a checklist worked to a large extent. It was found, though, that a number of results were missing, for instance with regard to water source and heating time. These were missed on account of the speed of the process and the difficulty in noting all points quickly. The water was provided in a beaker and the participants were asked to state where they normally acquired their water; this could be remedied in further work by the use of different water sources actually being available, for instance bottles and ampoules. This would most likely improve the recording rate for this category. The heating time was difficult to measure whilst still monitoring the participants' process. In a large number of cases the timing was lost owing to the interviewer forgetting to stop the watch as the procedure progressed into the filtering stage.

It is important to understand that this work is not of universal relevance. Heroin on

illicit sale in the UK is usually in the base form (King 1997). 'Crack' or freebase cocaine is the base form of cocaine. The base forms of these drugs are insoluble and require preparation for injection involving the application of acids. The addition of an acid to the base form of the drug forms the 'acid salt'. Heroin and cocaine in the acid salt form have very high water solubilities. This conversion enables the drug to be injected. In other countries, heroin is predominantly found in the hydrochloride salt form and therefore does not require the use of an acid during injection preparation. Crack always requires the use of an acid to prepare it, independent of location. The use of acids by crack/freebase cocaine injectors in geographical areas where heroin is sold in the soluble hydrochloride salt form would be of value for comparison to the results of this work.

Conclusions

These interviews enabled the characterization of the steps and equipment used by the participants to prepare their injections. The interview design succeeded in recording the required information.

This work provides information that has been unavailable before. Previous work has looked at the equipment used by injectors, including the water and acids, but the process of the preparation of an injection from insoluble bases has never been outlined, nor have the quantities used been recorded.

The preparation styles of the majority of the participants were very similar. This gives scope for the aims of improving the health implications from the injection of illicit drugs. The understanding of the injection preparation process is essential to the reduction of the harm that arises from injecting. If harm reduction workers focus on minimizing the harm involved in this technique, then the majority of users should easily be able to accommodate the benefits into their technique with little change.

The work highlights that practices such as injecting bottled mineral waters and the use of lemon juice still occur. It indicates that there is work still to be done in the field of harm reduction, and that these practices have still yet to be eliminated.

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